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IMMUNOLOGICAL RELATIONSHIP OF WHEATS RESISTANT AND SUSCEPTIBLE TO PUCCINIA RUBIGO-VERA TRITICINA[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 409

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Introduction

It is generally assumed that each individual organism is characterized by its specific protoplasm, and that degree of similarity of protoplasm is a criterion of genetic relationship. While the carbohydrates and fats (REICHERT **22, 23**) that occur as inclusions, or that enter into composition of the protoplasm of an individual display considerable specificity, the proteins seem to be the main factors in biological specificity (WELLS **28**). If one postulates evolution of organisms then one must postulate also an evolution of proteins. This may have involved new combinations of the 20 or so amino acids that are found to make up the protein molecule. ABDERHALDEN has calculated that 20 different amino acids could make 2, 432, 902, 008, 176, 640,000 different compounds, without including those that might be made by varying the proportion of the different amino acids in a single protein (WELLS **28**), and thus enable proteins to display variability of an order exhibited by organisms themselves. Immunological technique supplies a means of detecting protein identities, differences, and relationships which lie far beyond the range of the usual chemical technique. WELLS (**29**) points out that while the

[1] Space for the animals and experimental work was provided through the courtesy of Dr. W. H. TALIAFERRO, of the Department of Hygiene and Bacteriology.

biuret reaction will detect proteins in a dilution of 1:1000 or so, the precipitin test and the complement fixation test will detect proteins in dilutions of 1:10,000–100,000 and 1:1,000,000 respectively. Moreover, while the biuret test merely indicates the presence of a protein, the immunological test may tell which specific protein is present.

Immunological, or serological, technique exploits the fact that free proteins, such as egg white, and cells, such as bacterial thalli, apparently because of their proteins, can act as immunizing antigens when injected into the blood stream of suitable animals in proper dosages at proper intervals, engendering the formation of more or less specific antibodies. When these antibodies are brought into contact under suitable conditions with the protein used as immunizing antigen, or with other proteins called test antigens, they lead to specific reactions limited to, or more pronounced between, the immunizing antigen and its homologous antibody. Reactions with other proteins and the intensity of the reaction indicate more or less relationship between the protein of the immunizing antigen and of the test antigens. It appears that while the antigenic capacity of a protein depends upon the entire large colloidal molecular structure, its specificity seems to reside in certain of the radicals of the molecule. If a protein molecule possesses more than one reactive radical it may show more than one specific immunological reaction. Consequently group reactions exhibited by complex antigens from biologically related species may depend either upon the presence in these antigens of both common and specific proteins, or upon the presence in different proteins of common and specific reactive radicals. Even a single group within a radical may determine the specific immunological behavior of the entire molecule. Both the location and the character of the specific group are factors in determining specificity of the molecule. Specificity is apparently always a quantitative matter, reaching its maximum when the antigen is reacting with an antibody produced by immunizing with an identical antigen (WELLS **28**).

It has been shown that the globulin or prolamine fractions of plant proteins are highly potent and specific as antigens. Some of the most convincing chemical work of immunology was done with plant pro-

teins. OSBORNE (**19**) prepared various plant proteins as purely as possible, which WELLS (**18**) used in immunological tests, arriving at the conclusion that immunological specificity is dependent upon chemical individuality of the antigen. A study of the globulins of beans by JONES and WELLS (**29**) has demonstrated that the mung and adzuki beans are closely related to each other but distinct from other beans. LEWIS and associates (**12**) have demonstrated that the prolamines of emmer, einkorn, spelt, and durum wheats are closely related to gliadin and glutenin of *T. vulgare*, while those of teosinte and kafir are closely related to zein from *Zea mays*. No reactions were obtained between the proteins of the corn and wheat groups.

Serological technique has been used, not only to establish the identity of antigens or antibodies, as in detection of adulteration in meats, in flour, or meal of plant origin, or in bacterial infections, but it has also been used in phylogenetic studies, first for animals by NUTTALL (**15**), and later for plants in a less precise way by MEZ and his students (**13**). In the main the conclusions are in agreement with those based on comparative morphology, and when they are not, the serological evidence at times is given preference. The work of MEZ, however, has not been received favorably. MEZ (**14**) pointed out that when a serological test is made in phylogenetic studies, the protein reaction is in fact giving a clue to the relationship of the idioplasm of the organisms, and postulated that because of the great rôle of the nucleus in heredity, immunological tests give a clue to relationship of the nuclear proteins. If this be true, then on account of the rôle of chromosomes in heredity and phylogenetic relationship, it is possible that serological technique may be a means of studying the relationship of the proteins of the chromosomes.

It seems natural that serological technique should be applied as an aid in determining the systematic relations of economic plants in breeding work, and that geneticists have given heed to it as a possible means for determining invisible characters. ZADE (**30**) applied serological technique (specifically, the precipitin test) to determine the systematic relationships of oats and wheats. He pointed out that the morphological affinity as determined by appearance, and sexual affinity as determined by compatability in hybridization, are not reliable criteria in every instance. He found that the serolog-

ical relationships of oats and wheats are in good agreement with the relationships determined by morphological and breeding criteria. VAVILOV (**27**) pointed out that the groupings determined serologically by ZADE are in essential agreement with the groupings obtained when he used the criteria of resistance-susceptibility of wheats to *Puccinia rubigo-vera triticina* (red rust), and of anatomical and morphological characters in genetic and systematic study of these plants.

SAX (**21, 24**), in summarizing extensive cytological work by himself and others with wheats, points out that there is a striking association between chromosome number and morphological and other characters, such as adaptability to environmental conditions, disease resistance, quality of grain, and economic value. Chromosome number also plays a powerful rôle in sterility and genetic relationships. The einkorn group (haploid, 7 chromosomes) contains few varieties and is of little economic value. It is highly resistant to rust. The emmer group (haploid, 14 chromosomes) contains about 150 varieties and is of considerable value in semiarid regions. Its members are relatively resistant to rust. The vulgare group (haploid, 21 chromosomes) contains 500 varieties which are very adaptable, have proper quality of gluten for light bread, and are generally susceptible to rust. The increase in chromosome number and correlated increase in variability may be attributed to chromosome reduplication or increase in chromosome number through crossing, thereby offering opportunity for mutations to occur, and permitting the effect of a greater number of genetic factors (SAX **24**).

NELSON and BIRKELAND (**16**) used immunological technique to differentiate varieties within species, and demonstrated that the precipitin test, when conducted with the purified globulin fraction of wheat proteins, is of value to the geneticist in selecting hybrids for desired invisible characters, such as resistance of wheats to *Puccinia graminis tritici*. When any one variety is used as a standard, others can be classified serologically, the wheats showing the greatest number of genetic factors in common showing the closest relationship serologically.

Immunological technique has also been used to determine compatibility of individuals for grafting (DONTCHO **4**, GREEN **5**).

Investigation

This research was undertaken, at the suggestion of Professor GEORGE K. K. LINK, to determine the serological relationship of species, varieties, and hybrids, selected on the basis of their resistance to red rust, of the einkorn, emmer, and vulgare groups of wheats, and also to determine whether a parallelism or correlation exists between their ranking as determined by (a) immunological methods, (b) their chromosome number, and (c) their reaction to *Puccinia rubigo-vera* (Erikss) *triticina* (Mains and Jackson). The plan was to use representatives of the highly resistant einkorn (haploid, 7 chromosomes) group; representatives of the resistant emmer (haploid, 14 chromosomes) group; resistant and susceptible representatives of *T. dicoccum* (haploid, 14 chromosomes) which by some is taken to be the polymorphic progenitor of the susceptible and immune species of *T. vulgare* and *T. durum* (VAVILOV **26**); and susceptible, slightly resistant and resistant representatives of the *T. vulgare* (haploid, 21 chromosomes) group.

Because of the suspected but moot rôle of *Aegilops* species in the formation of the vulgare group, it was desirable to include a representative of this genus. PERCIVAL (**20**) contends that the vulgare wheats have arisen from a cross of *Aegilops* and a wheat of the emmer group, the *Aegilops* contributing, among other characters, susceptibility to rust. According to SAX (**24**), crosses of *A. cylindrica* and *T. vulgare* indicate that the two genera have one set of chromosomes in common. GAINES (**21**), on the basis of finding no paired chromosomes at reduction in crosses of *A. ovata* and emmer, suggests that there are 4 sets of chromosomes in the genera *Triticum* and *Aegilops:* einkorn containing chromosome groups *A* or *B;* emmer, *A* and *B; Aegilops*, *C* and *D;* and vulgare, *A*, *B*, and *C*. SAX (**21**) states that recent work of TSCHERMAK and BLEIER does not support this view. A fertile homozygous segregate from a cross of *A. ovata* and an emmer wheat was obtained. The F_1 hybrid had 28 pairs of chromosomes, due to doubling of the chromosome number in the first division of the fertilized egg.

Shortly after formulation of the early plans, Dr. E. B. MAINS, then at Purdue University Agricultural Experiment Station, was approached for suggestions as to suitable material. Dr. MAINS kind-

ly suggested that it would be well to include representatives of the vulgare group which show one type of response to rust in the seedling stage and another in maturity, and also a hybrid of the emmer (14) group and the vulgare (21) group which showed the resistance of the emmer group. This was of interest because SAX (**24**) apparently questions whether resistance can be transferred from varieties of *T. durum* to *T. vulgare* by crossing, stating that resistance frequently is dependent on the physiological condition of the hybrid. HAYES, however, points out that THOMPSON (**21**) was able to transfer resistance from durums to common wheats, and that he and his associates accomplished similar results. MCFADDEN (**21**) crossed *T. dicoccum*, resistant, a 14-chromosome wheat, with Marquis, a 21-chromosome wheat, and obtained a 21-chromosome plant, Hope, which is resistant.

The original plan had to be abandoned because it was found impractical to procure the desired forms[2] within each group, or to obtain sufficient quantity of seed to make the project feasible. From 500 to 1000 gm. of seed of each sample was needed to undertake the work, and it was therefore necessary to select those varieties of which a sufficient quantity of material was available.

The nine forms of wheat finally used in this research fall into three categories, based on their chromosome count, namely, the einkorn (7[3] haploid number), the emmer (14 haploid number), and the vulgare (21 haploid number) groups. Only one form was used in the einkorn group, and this was the most resistant of those tested. It is a double einkorn. In the emmer group three forms were used, one durum, and two emmers, and these were all leaf-rust resistant. Five forms were tested in the vulgare group, and these, the common wheat forms, varied from highly resistant to very susceptible to rust.

It was particularly unfortunate that but one form was procurable in the einkorn group, that no leaf-rust susceptible form was available

[2] In view of the fact that this study involved the use of both species and varieties, the term "form" is used to stand for species, varieties, or both. This is done to avoid repetition of the phrase "species and varieties."

[3] In the remainder of this paper the numbers (7), (14), and (21) indicate that the forms belong to the chromosome group represented by the numbers in brackets.

in the emmer group, and that no representative of *Aegilops* was obtained. These facts in themselves are sufficient to diminish somewhat the chances of securing the most striking results possible. Fortunately, however, through Dr. MAINS' kindness, a resistant hybrid (Hope, 21) of the emmer (14) and vulgare (21) groups was obtained, as well as a representative of *T. vulgare* which is very resistant as it matures, but which is susceptible to a number of physiological forms in its seedling stage.

TABLE I

GROUP	FORM	COMMON NAME	NO. OF WHEAT AND DESIGNATION	RESISTANCE RATING
1. Einkorn (7)	T. monococcum.	Einkorn	1* Ei	Very resistant
2. Emmer (14)	T. durum.....	Durum	3320 D	Resistant
	T. dicoccum....	Vernal emmer	1524 Em_1	Resistant
	T. dicoccum....	Emmer	293 Em_2	Resistant
3. Vulgare (21)	Hope.....	Common wheat	8178 Co_4	Highly resistant
	Marquis.....	Common wheat	3641 Co_3	Slightly resistant
	T. vulgare.....	Common wheat	2036 Co_2	Resistant in maturity; susceptible in seedling stage
	T. vulgare.....	Michigan amber	29 Co_1	Susceptible
	T. compactum..	Little club	4066 Cl	Susceptible

* Number under which the wheat was received from Dr. MAINS.

Table I summarizes the forms of wheat used in this investigation; the number under which they were received from Dr. MAINS; their grouping according to chromosome number; and their ranking on the basis of resistance-susceptibility to red leaf-rust as determined by inoculation experiments. Consequently, this research repeats in part ZADE's experiment, differing from it in that (a) genetically indexed species, varieties, and forms were used; and (b) purified globulin fractions were used (as in the NELSON and BIRKELAND (16) experiments) rather than the crude protein extracts used by ZADE. In this way it was hoped to differentiate between varieties which ZADE found difficult and impossible for oats when the varieties are of com-

mon descent. The paper is more comprehensive than that of NELSON and BIRKELAND, in that it involves representatives of the three great chromosome groups, and differs in that forms of wheat were selected on the basis of their resistance to *Puccinia rubigo-vera triticina*. Absorption tests were not made, however, as in the tests of NELSON and BIRKELAND. This would have added materially to the interest and significance of the results.

The globulin fractions of the protein of the grain of the varieties tested were obtained pure by electrodialysis, and then used as immunizing antigens by injection into rabbits. The precipitin test was used in which the criterion of reaction is the formation of a specific precipitate when immunizing antigen and antibody are brought into contact. The gross precipitate method first used was later abandoned for the more precise ring test, in which the lighter antigen solution is carefully layered in fine capillary tubes on the heavier antiserum in proper dilutions, and the precipitate appears as a ring at the interphase.

Method

The method involved four phases: grinding the wheat grains, dialysis of the extracted globulins, animal immunization, and serum titrations. From 400 to 1000 gm. of each form of wheat seed was put first through a coarse and finally through a fine mill. In every case the final product was fine enough to pass through a standard 100-mesh sieve.

DIALYSIS

The flour was first extracted in 300 gm. lots over a period of 12–18 hours in a 10 per cent sodium chloride solution, allowing 1 gm. of flour to 10 cc. of salt solution. At the end of this time the salt extract of soluble globulin was siphoned off, filtered through two thicknesses of fine toweling, and the filtrate dialyzed for 8–12 hours in an electrodialysis apparatus similar to that described by LOCKE and HIRSCH (9).

Two collodion bags of diameter (4.5 cm.) and length (16 cm.) were each fitted with rubber stoppers of the same bore, with a long inlet and short outlet of glass tubing for intake and output of distilled water. The stoppers were fitted with carbon poles. The col-

lodion bags were made from an 8 per cent solution of celloidin in a solution of equal parts of absolute alcohol and pure ether (BROWN **2**). These bags were molded in the inside of large glass tubes according to the method detailed by HAWK (**8**). After the ether had evaporated the bags were removed with water, dried, and then adjusted for suitable permeability for 12 hours in an 85 per cent alcohol solution (BROWN **3**). The bags were not allowed to dry after they had once been adjusted for permeability in alcohol. As soon as the bags were ready they were tied to closely fitting rubber stoppers and sealed fast with collodion. The inlet and outlet glass tubing and the carbon pole all had water-tight fittings. A pair of such bags was suspended and immersed in a museum jar of three or four liter capacity, containing sodium chloride extract of globulin. Iron clamps attached to the rubber stoppers and fastened to iron stands held the bags in place. A continuous flow of water from a single source was maintained through both bags, with just sufficient pressure to keep them distended.

The energy for electrodialysis was supplied from a direct current giving 110 volts in the primary circuit. By the introduction of a sliding rheostat, a secondary circuit was maneuvered which gave 60 volts. The dialyzer was started at 25 volts and gradually raised to 50 volts as the dialysis neared completion. A two-way switch was introduced into the set-up leading to the dialyzing apparatus, to afford a means for periodically changing the direction of flow of the current through the solution to prevent polarization.

After the supernatant liquid was siphoned off, the globulin precipitate so obtained was redissolved in 10 per cent sodium chloride solution, and diluted to 5 per cent and again dialyzed. A final purification of the globulin precipitate was made by dialysis from physiological salt solution. The purified globulin precipitate obtained after the third dialysis was filtered through hard filter paper, using a Büchner funnel and suction pump. The precipitate was washed on the filter paper with distilled water, and dried partially with the suction pump, and finally at 25° C. It was then pulverized in a sterile agate mortar and stored in glass vials. Each variety of wheat was treated in the same way.

IMMUNIZATION

Rabbits weighing approximately five pounds each were used for immunization. Each antigen was prepared in duplicate and six intravenous injections were given each animal at 4-day intervals. The immunizing antigen was dissolved in physiological salt solution (0.85%) to give a concentration of 0.005 gm. per cubic centimeter, and 1 cc. for the first injection and 2 cc. each for the other five injections were given as the immunizing dose. Before injection the immunizing antigen was just neutralized by the addition of N/20 NaOH.

Enough antigen material was prepared at one time for three injections, and this was held in the refrigerator for the interval of 8 days between the first and third injections. A second lot of antigen material was similarly prepared and stored at icebox temperature for the three other injections. Nine days after the sixth injection the rabbits were bled from the heart, and 25–30 cc. of arterial blood was drawn and placed in sterile flasks. When the clot formed the clear serum was transferred aseptically to sterile test-tubes and held in the refrigerator until such time as the precipitin titrations were run.

Two normal rabbits were held as controls and these were bled at the same time. Three days after the rabbits were bled, they was again injected with 2 cc. of 0.005 gm. per cubic centimeter of freshly prepared antigen and then bled on the eleventh day following the eighth and final injection. The second lot of serum was drawn aseptically from the heart, prepared, stored, and used in the final precipitin titrations. The results of the last test alone are reported in this paper.

Precipitin tests

During the second period of animal immunization, preliminary tests were made with the antiserum obtained at the end of the first period of immunization. All data recorded in this paper, however, are the results of the ring precipitin test made from titrations with the antiserum obtained after the second period of immunization.

Following NELSON and BIRKELAND (**16**), the mass precipitin test was first tried in these preliminary investigations. In this method regular Wassermann agglutination tubes were used and 0.5 cc. of diluted antiserum was pipetted into the tube to which 1 cc. of antigen

was afterwards added. A thorough mixing of the antigen-antiserum was accomplished by vigorously shaking the tubes, which were then allowed to stand at room temperature for one hour, read, and then incubated at 37° for 3 hours, again read at the end of the fourth hour, and held in the icebox overnight, when a final reading was taken for the quantity of precipitate deposited.

In all the mass precipitin titrations the test antigens were prepared at a concentration of 0.0005 gm. per cubic centimeter, and adjusted to neutrality with a solution of twentieth normal sodium hydroxide, the original pH of the antigen being 5.8. The antiserum was diluted with physiological salt solutions so as to give dilutions of 1–10, 1–20, 1–40, 1–80, 1–160, 1–320, 1–640, 1–1280, and 1–2560, and tested in these dilutions in every instance against its homologous immunizing and the heterologous test antigens. There was no apparent increase in precipitation after the first 4 hours, and hence the tubes were no longer held at icebox temperature. The small amount of precipitate and the difficulty of estimating quantitatively the amount of precipitate formed in the several dilutions made the task of reading the tubes difficult and the results unsatisfactory. In fact the results were so disappointing and conflicting that the method was abandoned after a fair trial with duplicate antiserum had been made.

In the final preliminary investigations, the ring precipitin test was used. In this test regular (bore 3 mm.) precipitin tubes were used. The test antigen was prepared in the concentrations already recorded and the antiserum in the same dilution as stated before. Enough antiserum was pipetted into the tube with a micropipette to fill it about one-third full, and then by the same procedure an equal amount of test antigen was carefully layered on to the antiserum. Several methods of incubating were tested, at icebox temperature, in a water-bath at 37° C., and also at room temperature. It was found that the best rings were formed within the first hour at room temperature; consequently all subsequent readings were made one hour after the layering of the antigen-antiserum, and then again as a check at the end of the fourth hour. It was further observed that the best results were obtainable when the test antigen was adjusted to pH 7 or to a pH slightly in the alkaline range.

The ring precipitin test gave definite results and indicated that the method was feasible for a study of the nine wheats. With these preliminary tests as a background, a more exhaustive and comprehensive study of the nine antigens was undertaken, and they were tested against their homologous and heterologous antisera obtained from the second immunization. This time the work was run in relays, three antisera in various dilutions against the nine antigens, until the lot of eighteen antisera were tested, always using the ring precipitin test.

TABLE II

TITRES OF PRECIPITIN TESTS

VERSUS ANTIGEN	ANTISERUM								
	Ei	D	Em_1	Em_2	Co_4	Co_3	Co_2	Co_1	Cl
Ei	[7]*	4	3	3	2–3	2	2	1–2	1
D	4	[7]	4	4	3–4	3	2–3	3	2
Em_1	3	4	[7]	3	3	2	3	2–3	2–3
Em_2	4	4	4	[6]	3–4	3	2	2	1–2
Co_4	3	4	3–4	3	[6]	2–3	3	2	3
Co_3	2	2	3	2–3	2–3	[5]	3	2	3
Co_2	1–2	2–3	3	1–2	2	3	[6]	2	4
Co_1	1	2	2	1–2	2	2–3	3	[5]	4
Cl	1	2	2	1–2	1–3	3	2	4	[7]

* The numbers recorded represent the titre of the test. Thus 1 represents a maximum titre in dilution 1–10; 7, in dilution 1–640; 4, in dilution 1–80; 2–3, in dilution ranging from 1–20 to 1–40, a definite decision between them being impossible. The numbers in brackets indicate the titres of the homologous reactions. The ranking of the antigens is arbitrary but on the basis of their chromosome numbers.

As a check, titrations of antigen-antiserum were run in which the antigen was diluted. The results were less definite than with the titrations of antigen-antiserum in which the antiserum was diluted. Titrations of antigens against normal serums, antigens against saline, and antiserums against saline all were uniformly negative.

The summarized data of table II show only the results from a single animal, since the duplicate tests with the second animal did not affect the final data to any significant extent. The numbers recorded in the table represent titres, titre being here used in the sense of highest dilution in which ring formation could be observed. At serum dilutions of from 1–80 to 1–160 the heaviest rings were obtained in the homologous reading. Table III shows the ranking of

the antisera on the basis of their titres and on the basis of their chromosome group relations, while table IV shows their ranking on the basis of their titres and their resistance-susceptibility relations.

TABLE III

RANK OF ANTISERUMS ON BASIS OF TITRES AND CHROMOSOME NUMBERS

CHROMOSOME NUMBER	VERSUS ANTIGEN	ANTISERUM								
(7).....	Ei	Ei*	[D*	Em_1	Em_2]	[Co_4	Co_3	Co_2	Co_1	Cl]
(14).....	D	[D	Em_1	Em_2]	Ei	[Co_4	Co_3	Co_1	Co_2	Cl]
	Em_1	[Em_1	D	Em_2]	Ei	[Co_4	Co_2	Co_1	Cl	Co_3]
	Em_2	[Em_2	D	Em_1]	Ei	[Co_4	Co_3	Co_2	Co_1	Cl]
(21).....	Co_4	Co_4	[D	Em_1	Em_2]	Ei	[Co_2	Cl	Co_3	Co_1]
	Co_3	[Co_3	Co_2	Cl]	Em_1	Co_4	Em_2	Co_1	D	Ei
	Co_2	[Co_2	Cl	Co_3]	Em_1	D	Co_4	Co_1	Em_2	Ei
	Co_1	[Co_1	Cl	Co_2	Co_3	Co_4]	[D	Em_1	Em_2]	Ei
	Cl	[Cl	Co_1	Co_3	Co_4	Co_2]	[D	Em_1	Em_2]	Ei
Ranking on basis of reaction.....		1	2	3	4	5	6	7	8	9

* Brackets indicate that members of a chromosome group form a serological group. Vertical order of arranging the antigens is that used in table II; the horizontal is according to titre.

TABLE IV

RANK OF ANTISERUMS ON BASIS OF TITRES AND RESISTANCE-SUSCEPTIBILITY REACTIONS

HOST REACTION	VERSUS ANTIGEN	ANTISERUM								
Resistant.....	Ei	Ei	D	Em_1	Em_2	Co_4	Co_3	Co_2	Co_1	Cl
	D	D	Em_1	Em_2	Ei	Co_4	Co_3	[Co_1]	Co_2	Cl
	Em_1	Em_1	D	Em_2	Ei	Co_4	Co_2	Co_1	Cl	[Co_3]
	Em_2	Em_2	D	Em_1	Ei	Co_4	Co_3	Co_2	Co_1	Cl
	Co_4	Co_4	D	Em_1	Em_2	Ei	Co_2	[Cl]	Co_3	Co_1
Resistant but susceptible in seedling stage.....	Co_2	Co_2	Cl	Co_3	Em_2	D	Co_4	[Co_1]	Em_2	Ei
Slightly resistant....	Co_3	Co_3	Co_2	Cl	Em_2	Co_4	Em_2	[Co_1]	D	Ei
Susceptible.....	Co_1	Co_1	Cl	Co_2	Co_3	Co_4	D	Em_1	Em_2	Ei
	Cl	Cl	Co_1	Co_3	Co_4	[Co_2]	D	Em_1	Em_2	Ei
Ranking on basis of reaction.....	.....	1	2	3	4	5	6	7	8	9

* Brackets indicate deviations from ideal ranking, ranging from invariably very resistant to resistant through variably or slightly resistant to invariably susceptible; or from invariably susceptible through slightly or variably resistant to invariably resistant or very resistant.

Discussion

Table II shows that in each instance the homologous antigen and antiserum gave the highest titre, indicating specificity of the reaction and individuality of each globulin. On the other hand, the group reaction of every antigen with every antiserum indicated that considerable relationship exists between the globulins of all the forms tested, or that all forms have some globulins in common. Tables II–IV show that serologically the members of the 14 chromosome group are closely related, and that they are related to *T. monococcum* (7). The members of the 21 group do not show as close a relationship among one another as do those of the 14 group. Some of them are more closely related to members of the 7 or 14 groups than they are to one another. For example, the highly resistant CO_4 (21) is more closely related to *T. monococcum* (7) and to D, Em_1, and Em_2 (14) than to CO_3 and CO_1 (21); CO_3 (21) is more closely related to Em_1 (4) than it is to CO_1 (21); and CO_2 (21) is more closely related to Em_1 (14) than to CO_4. On the other hand, CO_1 and Cl (21) in general show greater relationship to other members of the 21 group than they do to the 7 and 14 groups. All members of the 21 group excepting CO_4 are least related to *T. monococcum* (7).

It appears that the members of the 14 group are a homogeneous assemblage characterized by essentially the same globulins, and further that these globulins are similar to those of *T. monococcum* (7). This might be interpreted to indicate that the 14 group arose from the 7 group. That they are not identical might indicate the validity of GAINES' (**21**) contention that the wheats of the 14 chromosome group contain two sets of chromosomes, *A* and *B*, derived from einkorns with sets *A* or *B*. The members of the 21 chromosome group do not form so homogeneous an assemblage, greater differences existing in their globulins than exist among the globulins of the 14 group. Some of these are more like the globulins of the 7 and 14 groups than they are like those of other members of the 21 group. The globulins of all of the members of the 21 group save those of the highly resistant CO_4 are least like those of the one member of the 7 chromosome group.

Table III seems to indicate that serologically the resistant forms are closely related, and that in general they are more closely related

to one another and to the slightly resistant than to the susceptible forms. In general this applies also to CO_2, which is susceptible in the seedling stages but resistant in maturity. Practically ideal rankings are obtained when the resistant forms and the susceptible forms were tested against the others, the susceptible forms showing least relationship to the very resistant forms. When the slightly resistant form and CO_2 (which is very resistant when mature but susceptible in the seedling stage) were tested, it was found that a progressive ranking on the basis of either increasing resistance or increasing susceptibility cannot be made; in other words, their globulins are related to those of both resistant and susceptible forms, but in no regular way that could be detected by this method with the material at hand. This might be interpreted to mean that the globulins of the susceptible forms have come from forms other than the 7 or 14 forms tested. If *Aegilops* and a susceptible *T. dicoccum* had been available, a decisive answer might have been possible. The intermediate position of CO_2 is significant, indicating that it possesses globulins of the resistant and susceptible forms.

It would appear from this that the resistant forms are characterized by closely related globulins; that the susceptible forms are characterized by closely related globulins; and finally, that the slightly and varyingly resistant forms are characterized by globulins which are closely related to globulins of both resistant and susceptible forms. Furthermore, all the forms have some globulin in common, and each form is characterized by its specific globulin.

A comparison of tables III and IV seems to indicate that resistance and susceptibility of the forms studied are paralleled more closely by the presence of certain globulins than by chromosome number. Hope CO_4 (21), the resistant hybrid of an emmer (14) and Marquis (21) a slightly resistant form, shows greater relationship to members of the resistant 7 and 14 groups than it does to Marquis (21), its only moderately resistant parent. Marquis used as antigen shows about equal relationship to CO_2 (21), which is resistant when mature but susceptible when young, and to little club (21), which is susceptible, and to its resistant progeny CO_4 (21).

According to AAMODT (1), Hope is a hybrid produced by MCFADDEN by crossing vernal emmer and Marquis, a common wheat.

Hope is known to contain the chromosome number of the vulgare group, and is also the most resistant common wheat known, showing a resistance similar to that of its emmer (14) parent.

According to LEIGHTY (**10**), a high degree of association has been observed between emmer characters and rust resistance in segregation of crosses between emmer and common wheat, and likewise between durum and common wheats. Numerous crosses of these kinds have been made to secure rust-resistant wheats. From the thousands of segregates that have been grown, few have been obtained combining the good qualities of the vulgare group and the rust resistance of the emmer-durum group. Evidently Hope contains the chromosome number of common wheats and enough globulins peculiar to the emmer group to make it closely identifiable serologically with the 14-chromosome group. According to VAVILOV (**27**), the 21-chromosome group exhibits exceptional polymorphism, representing physiologically and morphologically a very heterogeneous group of forms.

The results seem to indicate that for the material used in these tests the serological technique will differentiate and group resistant and susceptible varieties, but that apparently it detects association between serological relationship and chromosome groups only in so far as chromosome number and resistance harmonize. VAVILOV (**26**) states that hybrids show that a strongly marked physiological immunity to rust is a dominant character, whereas in those forms showing little immunity, susceptibility is a dominant and immunity a recessive character; and that experiments have shown that immunity is not connected with distinct morphological characters but depends upon internal physiological peculiarities of the plant. If we substitute "serologically effective globulins" for the phrase "physiological peculiarities," we have an adequate statement of the serological behavior of Hope; and furthermore a support for the idea that the serological discrepancies of the partially resistant-susceptible members of the 21 chromosome group (which are neither completely rust-resistant nor wholly rust-susceptible) are associated with or due to the very nature of their mixed globulin constitution. Accumulation of chromosomes apparently does not increase resistance, although some resistant forms have a high chromosome count, namely, Hope (CO_4).

Conclusions

This research seems to indicate that globulins can be used as an index of resistance and susceptibility phenomena in the wheats studied, and that a closer parallelism exists between ranking on the basis of globulin relations and rust resistance, than on the basis of chromosome numbers and globulin relations. In view of the fact that resistance and susceptibility characters are genetically determined, their ultimate basis must be sought in the chromosome, specifically in the genes according to present views of geneticists. The questions then arise: Did the serological technique employed reach into the chromosomes to the genes? Are globulins of the chromosomes or of the genes involved in the resistance and susceptibility phenomena of the wheats studied? Positive answers are not inescapable. It is questionable whether the proteins studied specifically enter into the nuclear make-up to any extent (WELLS **28**). They probably are resident in the plasma.

All that the data seem to indicate, in the light of genetic experience with the forms tested, is that there is something (factor, gene) in the chromosomes of the resistant forms that determines the development of globulins which are in some way associated with or determine resistance, or that the factors which determine the formation of these globulins also determine resistance, or that the factors determining the formation of the globulins and the factors determining resistance are linked. These data also seem to indicate that some of the factors determining globulin formation are essentially the same, or come to expression in essentially the same way, in the resistant forms of the 7 and 14 chromosome groups. Consequently, the resistant forms of the 7 and 14 groups studied are closely related, and the 14 chromosome group may have arisen from the 7 group by reduplication; or by a cross of two einkorns with closely related but not identical chromosomes; or the 7 chromosome group may have arisen from the 14 chromosome group by loss of 7 chromosomes.

In the resistant forms of the 21 chromosome group some factors apparently are the same, or come to expression in the same manner as in the resistant 7 and 14 chromosome forms, and consequently may have been obtained from them by crossing, or in a mutation involving duplication. In the susceptible forms, however, this factor

or factors either are less active, or their action is balanced in some fashion so that a factor (or factors) for entirely different globulins come to expression. These globulins either are associated with or determine susceptibility, or their formation is determined by factors which also determine susceptibility, or their factors are linked with those determining susceptibility. Either these globulins are related closely enough to those associated with resistance to give serological group reactions with them, or the susceptible and resistant forms contain globulins of both kinds so that group reactions are obtained. At any rate the globulins associated with or determining susceptibility are of the same general nature as those associated with or determining resistance; and the factor determining formation of the former may have arisen by mutation from those determining the latter, or they may be of independent origin and therefore brought into the 21 chromosome wheats either by reduplication or by hybridization.

In the intermediate forms of the 21 chromosome group, both situations just stated for the resistant and susceptible forms exist simultaneously to a marked extent, but in varying degrees, so that globulins which are associated with or determine resistance, and globulins which determine or are associated with susceptibility occur simultaneously, or in succession in varying proportions in the same forms. It is unfortunate that a susceptible 14 chromosome wheat and a species of *Aegilops* which is susceptible were not available for this study as planned. In this manner some light might have been thrown on the nature of the factor determining the globulins which characterize the susceptible forms. Furthermore, some answer might have been reached as to whether the 21 group arose from the 7 and 14 groups by doubling and trebling of the chromosomes, or by hybridization with another form, such as *Aegilops*, which introduced a chromosome group carrying factors for susceptibility.

If the constitution of the globulins determines resistance or susceptibility, the relationship probably is not as direct or specific as that demonstrated recently by LINK, ANGELL, and WALKER (11) for protocatechuic acid and the resistance and susceptibility of the onion to *Colletotrichum circinans*.

This research seems to indicate, therefore, that serological technique may become an aid in systemtic and genetic studies of wheats.

In the forms studied it seems to be better adapted for differentiation and grouping on the basis of resistance-susceptibility characters, which are invisible characters that seem to have their basis in the constitution of globulin, or globulin complexes, than on the basis of visible characters such as chromosome numbers. One might be inclined to state that it is concerned more with so-called physiological than with so-called morphological characters, if it were not recognized that every morphological character also has its chemical basis.

This research gives promise that by the combined use of refined methods in separating the diverse protein constituents of wheat, and of more refined serological methods such as the complement fixation and the anaphylactic tests, as well as wise selection of forms of wheat and of *Aegilops*, a clue may be obtained as to the relationship of these wheats, and the source and distribution of the factors determining resistance and susceptibility.

Summary

1. Globulins of wheats of the einkorn, emmer, and vulgare groups were secured by electrodialysis in pure form and without denaturing, preliminary to use as immunizing and test antigens.

2. Relatively long periods of immunization (eight injections) of rabbits were necessary to obtain good titres in the precipitin ring test.

3. The reactions were highly specific in tests of homologous antigens and antiserums. Group reactions were obtained with the heterologous antiserums and antigens of all the wheats tested.

4. The mass precipitin test was not found satisfactory and usable.

5. The precipitin ring test gave precise and readily readable reactions.

6. A pH of 7 or one lying between pH 7 and 8.5 was necessary for the formation of rings.

7. Serological relationship seems to parallel chromosome number in the members of the 14 chromosome group studied. Parallelism between chromosome number and serological relationship does not seem as definite for the forms of the 21 chromosome group studied; some forms standing closer to forms of the 14 chromosome group than to other forms of the 21 chromosome group.

8. Serological relationship of the various forms of wheat tested

seems to be closely paralleled by relationship as measured by their resistance-susceptibility behavior to *Puccinia rubigo-vera triticina*.

9. The relationships determined immunologically by use of globulins as immunizing and test antigens seem to be more in harmony with relationships determined by breeding and inoculation, using resistance to *Puccinia rubigo-vera triticina* as the criterion, than with relationships determined cytologically, using chromosome number as the criterion.

10. The precipitin test and more refined immunological tests, using pure proteins as antigens, give promise of furnishing a clue to the relationship of wheats, their derivation, and the source and distribution of the factors that determine resistance and susceptibility.

I wish to express my thanks to Professor GEORGE K. K. LINK, of the Botany Department of the University of Chicago, who suggested the problem, for continued interest and valuable criticism during the research, and to Dr. E. B. MAINS for material and helpful suggestions relative to selection of the material used in this research.

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ECOLOGICAL STUDIES OF THE VEGETATION OF THE GREAT SMOKY MOUNTAINS OF NORTH CAROLINA AND TENNESSEE

I. SOIL REACTION AND PLANT DISTRIBUTION

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 410

STANLEY A. CAIN

(WITH SIX FIGURES)

Introduction

The Great Smoky Mountains National Park, in the North Carolina-Tennessee region of the Southern Appalachians, offers an excellent field for the investigation of soil reaction in relation to plant associations. It is in these mountains of high rainfall and luxuriant vegetation that the greatest height east of the Rocky Mountains is reached. On these lofty peaks, many of them rising over a mile in altitude above their immediate base, is found a forest rich in numbers, in species, and with a magnificent development of individuals.

Procedure

In most instances soil samples were taken in pairs, one at the surface and one immediately beneath, at a depth of 6 inches. A surface sample consists of 200 gm. or more of soil taken immediately beneath the duff. This stratum of the soil is considered important as the level of germination of seeds. The sample from a depth of 6 inches represents in most instances what may be called subsoil. In the heath bald and spruce-fir associations this depth was frequently insufficient to pass below the peaty layer, although samples from the subsoil were often equally acid. It is assumed that the 6-inch depth represents the medium of establishment of seedlings, while it is known that for many species this is about the level of greatest root development. In the pine heath the soil was frequently of little greater depth than 6 inches. In the peaty soils of birch woods, heath bald, and spruce-fir woods, woody plants were found to be very shallow rooted, while herbaceous plants were strikingly rare. What-

ever the relation of the reaction of the soil to the vegetation may be, such samples are probably adequate for pH studies of plant communities.

All determinations of the hydrogen-ion concentration of soil samples were made electrometrically on the Youden hydrogen-ion concentration apparatus. This portable apparatus was put on the market early in 1928, and was used in the field by the writer in preliminary investigations that spring. Subsequent tests, in 1928 and 1929, were made in the laboratory on soil samples which were brought in, air-dried, and tested at the earliest convenience. It has been shown by GUSTAFSON (**8**) and others that soils so treated do not show appreciable change over considerable periods of time. (Although the Youden apparatus is convenient for field work, the ability to collect soil samples and read them several days or even weeks later in the laboratory is more convenient, eliminating the carrying of equipment.) From ten to sixty samples were taken in each of the associations studied, selected from different locations in the same stand and from stands as many as 40–50 miles apart. Altogether 289 soil samples were determined for hydrogen-ion concentration. Three separate tests (separate soil solutions) were made on each soil sample collected, to determine the extent of very local variations.

It was found that the average deviation of three tests each for 220 samples was 0.21, or approximately 0.1 pH from the mean of the three tests. When the samples were considered in regard to their degree of acidity, it was found that the deviation averaged about the same anywhere between pH 3.5 and neutral. For example, the average of 11 samples, lying between 3.5 and 3.9 was 0.2 deviation; for 54 samples, lying between 5 and 5.4 was 0.18 deviation; and for 10 samples, between 6 and 6.4 was 0.21 deviation. On a basis of this work, some by CAIN and FRIESNER (**1**), and some unpublished data, totaling about 2000 tests, it is concluded that one test per sample is sufficient. In the first place, different soil samples may show a greater divergence; while such minor variations cannot be interpreted in relation to plant distribution.

The determination of acidity by the Youden apparatus permits a ready translation of the e.m.f. into pH, the common form of expres-

sion. In certain earlier work the writer, together with other investigators, has made the mistake of overlooking the logarithmic nature of the pH numbers in performing such operations as arithmetical averaging of pH determinations. WHERRY (20) has presented a valuable discussion of common mistakes in manipulation of pH data, and, primarily for the layman, a more readily understandable method of stating acidity and alkalinity. He proposes the term "active acidity" as the amount of hydrion present in the solution free to exert the effects commonly classed as acidity. WHERRY explains the concept as follows:

> The amount of hydrion present in a liter of pure water is taken as the unit of acidity; it amounts to 0.0000001 gram. By use of a table of logarithms, the quantity of these units represented by each successive pH number is calculated. From the total amount of hydrion thus calculated is subtracted the amount of hydroxylion which is also present, the one being the reciprocal of the other, and the result is rounded off to the nearest 0.5.

In the study of the acidity of soils of various associations in the Smoky Mountains, the results have been expressed in range in pH, and also in "active acidity" in which the logarithmic relations are more apparent. Averages are expressed in pH because of the greater familiarity of the term. These pH numbers were obtained by changing each pH determination into its equivalent "active acidity," averaging the results arithmetically and changing the average back into pH. The importance of this step is apparent when we consider the results of incorrectly averaging pH numbers arithmetically and by active acidities, when it is found that the discrepancy is frequently as much as 15 to 20 per cent.

Preliminary results

With the work of SALISBURY (17) on the relation of pH to topography in mind, it was decided to investigate that aspect of soil reaction and vegetation in the Smoky Mountains. This seemed a promising field because of the characteristic associations, here called heath balds, which are common on the ridge tops and upper slopes at the higher elevations in the mountains. Since this association is dominated by members of the Ericales, which are notoriously acid-tolerant, and since the lower slopes and coves are occupied by com-

mon woodland types, it was thought that the pH relations to the vegetation and topography might be discerned. A line was selected which would pass from Brushy Mt. to Ball Mt., cutting across two sharp ridges between. The tops and the upper southwest slopes of these four prominences are occupied by heath bald associations. High on the north exposures and lower on the southern, the balds give way to chestnut-oak woods, and lower in the valleys to the cove type of forest (FROTHINGHAM 6). Along this line, soil samples were selected at intervals, the acidities of which are indicated in fig. 1. It will be seen that on these four prominences, and on two

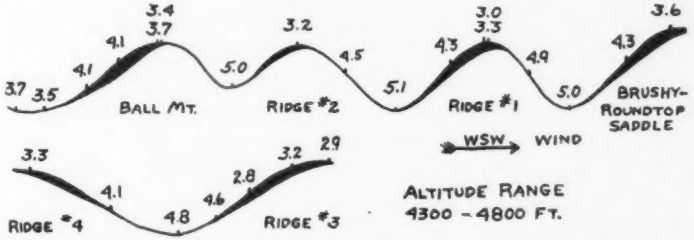

FIG. 1.—Two lines of soil samples taken across ridges to show position of heath bald associations and altitudinal relations of soil acidity (expressed in pH numbers at point where samples were taken); vertical scale is grossly exaggerated.

others near by, there is a rather characteristic distribution of the heath balds, as indicated by the broad black portions of the line. It is also apparent that, in so far as these few readings are indicative, the ridge tops are highly acid, the coves moderately acid, and the middle slopes intermediate in acidity as well as in position. Although the heath balds are generally higher in acidity than the woodland associations, it will be seen that the reaction is not related to vegetation alone, but in part at least to topography. Similar relations have been worked out for hills 100–200 feet high by CAIN and FRIESNER (1). With the stimulus derived from these preliminary results the investigation was carried on later in 1928 and 1929.

Associations

The soil samples determined for acidity have been arranged for the purpose of this study by major associations, which in turn have

been considered from the point of view of their altitudinal distribution. The following is a brief description of the associations studied.

JUNIPER WOODLAND.—Soil samples for the juniper woodland study were taken a few miles south of Knoxville, Tennessee. These soils are usually shallow, rocky, and strongly alkaline. The vegetation of this association has recently been described by PICKLESIMER (13) as the so-called cedar glades, near Nashville, Tennessee. *Juniperus virginiana* is dominant, and *Ulmus alata* and *Celtis mississippiensis* are the most important associates. *Symphoricarpus orbiculatus* and *Adelia ligustrina* are common shrubs, while *Sedum pulchellum*, *Arenaria patula*, *Erigeron ramosus*, and *Geum canadense* are common herbs. In the comparison of soil reactions with altitude, the juniper woodland cannot be considered with the rest of the associations since it is the only one with limestone bedrock.

FALLOW FIELD.—In the vicinity of Gatlinburg, Tennessee, at the foot of Mt. LeConte, which is the locus of most of this investigation, a fallow field was selected to represent the soils at the low altitude of approximately 1200 feet. This field has not been modified by application of fertilizers. The disturbance of the soil by cultivation in previous years has resulted in a more homogeneous profile, and possibly a slightly less acid condition than would otherwise have resulted.

CHESTNUT WOODS.—The soil reaction of two different stands of chestnut type were investigated from samples taken in the vicinity of 3000 feet altitude. *Castanea dentata* predominates, with a varying admixture of *Quercus rubra*, *Q. prinus*, *Fagus grandifolia*, *Acer saccharum*, *Halesia carolina*, *Carya cordiformis*, *C. laciniosa*, *Cercis canadensis*, *Robinia hispida*, *Sassafras variifolium*, etc. Elimination of the chestnut by the ravages of blight is converting this into the chestnut-oak type (FROTHINGHAM 6). The chestnut woods is a common type occurring frequently on dry north-facing slopes, and south-facing slopes to an altitude of almost 5000 feet in places. The soils are sandy and frequently shallow, and toward the higher altitudes may be semipeaty at the surface, and from subacid to low mediacid in reaction. Frequently on lower ridges the south-facing slopes are occupied by pine woods, while the north slopes at the same altitude are covered with chestnut.

PINE HEATH.—The "pitch pine-mountain pine type" of the foresters is one of the least valuable forest types, but is very interesting. Its most abundant species in the region of these studies are *Pinus echinata* and *P. pungens*, which occur in pure or mixed stands. Other associates of these are *P. rigida*, *P. virginiana*, *Robinia pseudoacacia*, *R. hispida*, *Acer rubrum*, *Nyssa sylvatica*, and *Castanea dentata*. The shrubs and ground cover are particularly interesting, with members of the Ericaceae most striking: *Kalmia latifolia*, *Pieris floribunda*, *Vaccinium* spp., *Gaylussacia ursina*, *G. resinosa*, *Gaultheria procumbens*, *Epigaea repens*, etc. Among the herbs are *Pteridium aquilinum*, *Aletris farinosa*, and the orchids *Pogonia divaricata*, *Calopogon pulchellus*, and *Habenaria ciliaris*. The Compositae of the late summer season have strong southern affinities (KEARNEY 9), but as a whole this community is striking in its close resemblance to the pine-barren flora of the Atlantic coastal region.

On Tower Hill, Piney Mt., and similar locations the soft woods occupy the southern exposures, and the line of demarcation between them and the previously mentioned chestnut woods is frequently as sharp along the crest of the ridges as if the two associations were planted. Complete transition may occur within the space of two or three meters.

COVE FOREST.—The moist slopes and cove forests are divided into a number of types, according to the species or groups of species occurring as dominants. The present soil samples were taken from the valley of the Roaring Fork, on Mt. LeConte, at about 4000 feet elevation, where the following species are characteristic: *Tsuga canadensis*, *Betula lutea*, *Aesculus octandra*, *Halesia carolina*, *Liriodendron tulipifera*, *Fagus grandifolia*, *Acer saccharum*, *Quercus velutina*, *Prunus serotina*, etc. Undershrubs include an abundance of *Rhododendron maximum*, *Leucothoe catesbaei*, *Ilex opaca*, etc. Toward the upper parts of the coves, at elevations of about 5000 feet, the northern hardwoods are frequently found in pure stands of one, or at the most two or three species. The soils are loams, usually of considerable depth except on the steeper slopes, and are in the main low mediacid, although sometimes they reach low superacid reactions.

BIRCH WOODS.—Practically pure stands of *Fagus grandifolia* or of *Betula lutea* and *Aesculus octandra* are commonly found at altitudes

of 4500–5500 feet, particularly in saddles and upper cove heads, but *Betula lenta* infrequently constitutes one of these consociations. The outstanding peculiarity of the *B. lenta* forest here is the formation of raw peat at the surface of the soil. In so far as the writer knows, this is the only deciduous forest in the Great Smoky Mountains with raw peat formation. Here the formation of peat may be attributed in some way to the extreme abundance of evergreen undershrubs, of which *Rhododendron maximum* and *Leucothoe catesbaei* are commonest.

BEECH ORCHARD.—In the Great Smoky Mountains the subalpine forest is predominantly spruce-fir, but certain types of northern hardwoods can be classified as subalpine when the trees show distinct dwarfing and distortion from the influence of the factors concomitant with altitude. Such woodlands are common in certain portions of the southern Appalachians, notably in the Craggy Mountains, Black Mountains (DAVIS 3), and in the Smoky Mountains. In so far as the writer has been able to learn, this type of "orchard" woodland is not found elsewhere. The name is of local origin, in that the natives frequently refer to "beech orchards" or "chestnut orchards" when speaking of woodlands of stunted form at high altitudes (figs. 2, 3). Excellent examples of this form are to be found in the southern part of the Great Smoky Mountains, on Thunderhead Mt. and peaks and ridges southward to the Little Tennessee River, along the North Carolina-Tennessee state line. These mountains are topped by grassy balds (subalpine meadows). Toward the upper tree limits, about 4500–5000 feet, according to the exposure the trees become dwarfed, distorted, and wider spaced. In extreme cases the boles are short and stocky, with spherical crowns, attaining a total height of 10–20 feet. Reproduction in this type is predominantly, if not exclusively, by sprouting.

Farther north in the Smoky Mountains, starting with Clingman's Dome Mt., the peaks rise 1000–1500 feet higher than in the southern part of the range. Here the upper slopes are covered with magnificent forests of virgin spruce-fir. In the gaps between peaks and on the upper slopes of coves, beneath the spruce-fir, the "orchards" of northern hardwoods recur at about the same altitude as in the southern region of the grassy balds, although they do not show as striking form changes.

Soil samples for the present study were taken from beech consociations related to both the grassy balds and the spruce-fir, some 40–50 miles apart. The reactions show a striking similarity through-

FIGS. 2, 3*.—Fig. 2, orchard woodland of chestnut, red oak, beech, and yellow birch, a subalpine type found near the margins of grassy balds; fig. 3, pole stand composed mainly of *Abies fraseri*, on Mt. LeConte at about 6500 feet elevation.

*All photographs used in this paper are copyrighted by J. Thompson Co., Knoxville, Tenn., and used through their courtesy.

out both these stands. There is absolutely no peat formation, and a complete absence of Ericaceae, although they are present in contiguous associations.

The northern hardwoods (and the beech will serve to illustrate as well as any) found scattered in the lower coves are magnificent specimens, quite equaling if not surpassing anything found in the north where they are intraneous. The pure stands of beech in the subal-

pine, however, are frequently so changed in aspect that they are hardly recognizable as the same species.

CHESTNUT ORCHARD.—The orchard type has been sufficiently considered in the preceding section. The members of the chestnut orchard are almost exclusively *Castanea dentata*, *Quercus prinus*, *Q. rubra*, and *Aesculus octandra*. The orchard woodland is formed from the cove forest by a gradual transition from the similar but floristically richer associations of lower altitudes.

FIG. 4.—Grassy bald at about 5000 feet elevation, in southwest part of Great Smoky Mts.

GRASSY BALDS.—These treeless areas (balds) on the peaks and higher ridges of the southern Smokies are conspicuous features of the landscape (fig. 4). As has been stated, the trees begin to show striking form changes at about 4500 feet, and are completely eliminated 100–200 feet below the top, forming the mountain pastures or "alps." A number of native and introduced grasses, abundant ruderals, and a few low shrubs compose the flora. The influence of over a hundred years of intermittent grazing has made its impression on the vegetation; but in all probability these balds are natural phenomena. (This problem will be dealt with in a subsequent paper.) It is sufficient to mention that soil profiles show from a few inches

to a foot or more of homogeneous black soil of grassland type, which is too deep and mature to have developed since the advent of the white man, with the possibility of his having cleared off the trees. The eccentric growth rings of trees situated at the margin of the balds indicate that many of them have been so situated for nearly a century, although their stature is about 12–15 feet. Even had the early settlers cleared these blads, that fact would be an indication of the previous existence of open savannah woodlands with native potentiality for grassland, with slight climatic change.

SPRUCE-FIR FOREST.—This formation constitutes the majority of the subalpine forest and, as would be expected, is definitely northern in its floristic affinities. About 60 per cent of its woody flora is extraneous, the species finding the bulk of their ranges in northeastern United States, southeastern Canada, while some of them are transcontinental in high latitudes. In the last two categories are *Picea rubra* and *P. mariana*,[1] while the third dominant of this coniferous forest is an endemic, *Abies fraseri*. Endemics of narrow range and circumscribed habitat, in the southern Appalachians, constitute 27 per cent of the woody flora, including some beautiful ericaceous shrubs, as *Rhododendron catawbiense*, *R. punctatum*, *Dendrium prostratum*, and *Hugeria erythrocarpa*.

Of the conifers, Fraser's fir particularly shows a tendency to stagnate in pole stands at higher altitudes. The soil shows a decided podsolization, and is highly acid, being practically superacid throughout. The basin or "sag" on Mt. LeConte, at approximately 6500 feet, is a good example of this type of stagnant pole stand. The surface of the soil profile shows peat formation from an inch or so to over a foot in thickness, with a leached layer beneath. The ground cover consists of a dense carpet of ferns of the genera *Asplenium* and *Aspidium*, with *Oxalis acetosella*, *Clintonia borealis*, *Trillium undulatum*, and an almost continuous cover of lichens and mosses, among which *Sphagnum* species are particularly abundant.

HEATH BALD.—The so-called heath balds or "slicks" of the natives are treeless areas (fig. 5) which occur mainly as inclusions in the spruce-fir, but are also found in the region of the northern hard-

[1] Although *P. mariana* has been reported from the region, there is some doubt as to its presence in the Smokies.

woods. The physiognomy of these balds can best be described as broad-sclerophyll scrub formations; but the terms heath bald or ericad bald have a certain value, in that they suggest the importance of members of the Ericaceae. These areas are completely dominated by members of this family, which make up 40–50 per cent of the woody flora. Herbaceous plants are strikingly absent. The present tests were made from soil samples taken at about 5000 feet altitude, although some taken at about 6600 feet are considered. The under-

FIG. 5.—Heath bald in Rocky Spur region, Mt. LeConte, at about 5000 feet elevation.

lying rocks are sandstones, sand conglomerates, and quartzites, that have been much crushed and folded. The residual soils are thin and coarse, and run mediacid in reaction. On top of this subsoil is a peat deposit varying from a thin dry tundra-like crust to three or four feet of wet brown peat. In many places, particularly at the higher altitudes, the substratum is so damp and the atmosphere is so constantly humid that *Sphagnum* and associated mosses cover the ground, while *Usnea* and similar lichens are draped over the branches of the low shrubs. Although they are more characteristic of the highest peaks, the heath balds occur as low as 4000 feet in altitude, where the edaphic conditions are suitable.

Results and discussion

RELATION OF ACTIVE ACIDITY TO ALTITUDE.—In 1922 SALISBURY (17) advanced the opinion that "woodlands in general and probably all types of undisturbed plant communities in this country [England] are tending to become progressively more acid with consequent changes in the character of the vegetation." Furthermore, he concludes that there is more active leaching on high than on low ground, resulting in a depression of the upper limits of the zones as the summit vegetation extends with the extension of higher acidity. In this process of soils becoming more acid, the leaching out of bases, of which calcium carbonate is most important, increases the potentiality for acidity since bases become insufficient to neutralize the acids produced. WOODHEAD (21), in a recent consideration of the degeneration of vegetation types on the Pennine plateau, states:

> With a high rainfall leaching would go on and the soil, naturally poor in bases would become still more deficient, not only in lime, but in other alkaline bases. Here we have the beginning of those changes which not only retard and ultimately stop the decomposition of humus, but an acid condition accompanies the increase in organic matter.

In a recent extensive study of soil acidity in the forest, FRANK (5) has brought out the fact that sites above 700 meters (in the Black Forest near Freiburg) are generally more acid than corresponding sites at lower altitudes; that ridges, peaks, and exposed sites tend to become acid; upper slopes are more acid than lower slopes; and that in the soil profile, acidity tends to vary vertically as well as horizontally. In undisturbed soil profiles the highest acidity would be expected at the surface, becoming progressively less acid at greater depths, in direct relation to the extent of leaching out of bases. This phenomenon has been demonstrated adequately by SALISBURY (17), STICKEL (18), and CHRISTOPHERSON (2). In a *Vaccinium myrtillus-Betula pubescens* forest the latter finds that the raw humus layer is pH 4.0; the leached layer, 4.1; and the enriched layer, 4.5. Again, in a *Calluna-Cladonia* heath the three layers are respectively pH 4.3, 4.4, and 4.7.

In the present work, in 110 sets of surface and subsoil, taken as described at the beginning, the surface reactions averaged 0.2 of a pH more acid than the subsoils. The results of the investigation are

shown in table I, giving ranges and averages in both pH and active acidity equivalents, grouped according to plant associations, which in turn are arranged according to altitude.

The relations between surface and subsoil samples may be illustrated by a few examples. In the chestnut woods in one profile the surface soil had an active acidity of 40 (pH 5.4), and the subsoil an active acidity of 12.5 (pH 5.9). In the birch woods one profile was, surface 800 (pH 4.1), subsoil 250 (pH 4.6). While in the grassy balds many samples were the same, as both surface and subsoil 160 (pH

TABLE I

ACIDITY OF PLANT ASSOCIATIONS ARRANGED ACCORDING TO ALTITUDE

ASSOCIATION	ALTITUDE	AVERAGE REACTION				RANGE IN REACTION			
		Surface soil		Subsoil		Surface soil		Subsoil	
		pH	Active acidity	pH	Active acidity	pH	Active acidity	pH	Active acidity
Heath bald	6600	3.2	6300	3.8	1600	.....	.....	.....	.....
Spruce-fir	6500	3.6	2500	3.8	1600	4.3–2.9	500–12500	4.4–3.0	400–10000
Heath bald	5000	3.5	2980	4.0	980	4.1–2.8	867–16000	4.5–3.5	315–3150
Grassy bald	5000	4.8	169	4.9	141	6.0–4.3	9–473	5.9–4.5	13–293
Beech orchard	4700	4.5	291	4.5	293	4.8–4.2	160–587	4.7–4.4	173–383
Chestnut orchard	4700	4.6	226	4.6	241	5.0–4.4	108–372	4.7–4.5	187–315
Birch woods	4400	4.2	607	4.4	367	5.0–3.8	100–1733	4.7–4.4	200–400
Cove forest	4000	4.2	630	4.6	250	5.1–3.5	80–3150	4.9–4.4	125–400
Pine heath	3400	4.9	140	5.0	114	5.6–4.1	28–800	5.8–4.5	16–315
Chestnut woods	3000	5.3	55	5.3	51	6.8–4.5	1–321	5.9–4.8	12–161
Fallow field	1200	5.9	13	6.0	11	6.3–5.5	5–31	6.4–5.6	4–25
Juniper woodland	1000	7.9	8*	7.9	8*	7.7–8.1	5–12*	7.7–8.0	5–11*

* These nos. are in terms of "active alkalinity."

4.8), occasionally the surface soil was less acid, as in the beech orchard where one sample showed 315 (pH 4.5) and subsoil 500 (pH 4.3).

Since the influence of leaching is important in increasing acidities of upper horizons of soil profiles, the accumulated effect should be apparent in regions of uneven terrane, that is, hilly and mountainous country. More rapid leaching on high than on low ground would result in altitudinal depression of acidity zones. In the Sycamore Creek region, Indiana, CAIN and FRIESNER (1) have shown certain conformity between reaction and topography of hills about 100 feet high. Hydrogen-ion curves parallel topography curves of the ridges, the former ascending (more acid) with the latter (higher altitude).

CHRISTOPHERSON (2) reports on ten subalpine associations and five alpine associations in Sylene National Park, Norway, having a range from pH 3.6 to 7.1, but does not bring out the altitudinal relations. In the Great Smoky Mountains, however, it is possible to observe striking relations between soil acidity and altitudes, ranging from 1200 to 6600 feet. Referring to table I, we see an essentially progressive increase in soil acidity with increased altitude. This is illustrated graphically (fig. 6) for both subsoils and surface soils.

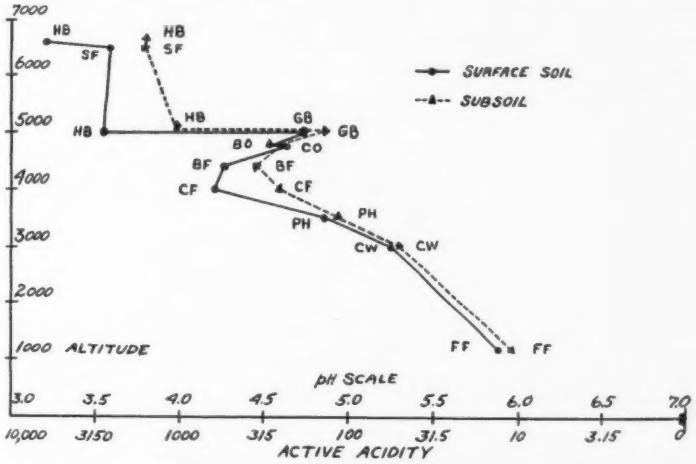

FIG. 6.—Curves constructed on basis of average reaction of plant associations plotted against altitude; symbols of associations are (from top to bottom); *HB*, heath bald; *SF*, spruce-fir forest; *GB*, grassy bald; *BO*, beech orchard; *CO*, chestnut orchard; *BF*, birch forest; *CF*, cove forest; *PH*, pine heath; *CW*, chestnut woods; *FF*, fallow field.

Assuming an altitudinal scale, that is, that the factors concomitant with increased altitude increase soil acidity, other things being equal (and they are not), those associations which are too acid for their relative altitudinal position show peat formation at the surface of the soil (as in the birch woods), or have an abundance of evergreen undershrubs belonging to the Ericaceae (as in the birch woods and the cove forest). On the other hand, those associations less acid than a curve of increasing acidity with altitude would indicate are strictly deciduous, even to their ericaceous elements (as the

grassy balds and chestnut woods, including the orchard type), or are entirely without ericads (as in the beech woods). On the whole, as indicated by the curves (figs. 1, 6), the increase in acidity of both surface and subsoils with altitude is striking, and certainly represents, among other things, the influence of long continued leaching.

In all associations but the beech and chestnut orchards the surface is more acid than the subsoil, while they are strikingly homogeneous, as indicated by the convergence of the lines in fig. 6. It will be noticed that the associations with upland peat formation (birch woods, heath balds, and the spruce-fir forest) show greater differences between the surface and the subsoil acidities, as well as greater average acidity. Of course the leaching out of bases is not the only factor in the varying acidity of the soil profiles. SALISBURY, quoting FLICHE and GRANDEAU, states:

> The basic content of deciduous foliage retards the leaching process for a time, the supply of salts which the foliage contains will decrease as the soil becomes more leached till ultimately the species originally present becomes replaced by others more tolerant of acid conditions, which return an almost negligible amount of salts to the soil.

In this connection it may be observed that the associations of an entirely deciduous nature (chestnut woods and orchard, beech woods and grassy bald) are generally less acid than those with important evergreen elements.

HIGH ACIDITY PROBLEM.—The high acidity of all the mountain associations here considered requires a consideration of the factors involved, particularly since some of the associations show acidity higher than has previously been reported for plant associations in the United States. The culmination is reached in the subalpine heath balds, ericaceous communities which are here first recognized in American ecology (CAIN **22, 23**).

The first factor to be considered is the nature of the underlying rocks. All the associations considered above 3000 feet are on sandstone, sandstone conglomerate, quartzite, or slate, all of which produce inorganically acid soils, usually around the mediacid portion of the scale, pH 4.4–4.6. The acidities of the various associations (leaving out the juniper woodland) may validly be compared and altitudinal differences considered, as related to leaching and addition of bases, etc.

The great age of these mountains, the oldest in North America, is an important factor in the production of high acidities of the soil, on the basis of the long duration of a number of interrelated processes. High rainfall, high humidity, and low temperature combine to provide climatic and edaphic conditions of primary importance in the development of soil acidities. There is no weather bureau anywhere in the Great Smoky Mountains, and data from the adjacent valley country, as Knoxville, Asheville, etc., are of little value. GALYON (7), however, in a study of the woody plants of east Tennessee, reports a rainfall of 83 inches for the higher slopes of the Smokies. The great humidity so characteristic of these mountains finds expression in their name, because of the almost constant fogs and clouds which rise from the damp coves, obscuring the upper slopes. One of the most apparent results of the climate is the luxuriant vegetative growth covering every available foot of surface, a rich, dense, and practically unbroken wilderness, unlike anything else in North America. This is the forest center of eastern United States and Canada, including, as it does, remnants of the ancient and once widespread Miocene flora, and the center of distribution of postglacial forests.

The climatic conditions and the luxuriant vegetation combine in the production of upland peat. The high moisture content of the soil results in low temperatures, poor aeration, and low microorganic activity, favoring poor decomposition of the vegetable litter. At the same time these factors favor the growth of *Sphagnum* and other peat-forming mosses and ericaceous shrubs and conifers capable of growing under such climatic and edaphic conditions (WOODHEAD 21). That decomposition of ericad and conifer leaves tends to produce high acidities and podsolization is well known. This has been described for pure white pine stands in New England by FISHER (4) and by STICKEL (18). That *Sphagnum* and certain other mosses tend to increase acidity of the medium in which they grow has been indicated by KURZ (10). The probability is also high that such is the case for most of the Ericaceae; that they are a factor in acidity increase, per se.

BIOLOGY OF HEATH PLANTS.—The importance of the "calcifuge" or acid-tolerant plants, in this case primarily Ericaceae, in the zones of highest acidity is apparent, and although the biology of these plants

has not been investigated by the writer, one or two of the factors involved in their success may be mentioned. It is not within the scope of this paper to discuss the establishment of the heath balds or the problems connected with the question of xeromorphy, a field reopened recently by MAXIMOV (**11**). It is pertinent to mention the work by THATCHER (**19**), however, who has shown that whenever a plant is able to produce a healthy root system in peat it is also able to obtain sufficient water from the peat to maintain a high rate of transpiration, in fact "a higher rate of transpiration than that maintained by the same plant grown under identical conditions in a loam soil." The question of "physiological drought" does not seem to exist for these plants, however; the species capable of establishing root systems in peat are few and are practically all woody and conspicuously ericaceous.

Combine with this the facts that the Ericaceae are conspicuously symbiotic with mycorhizas, and that the biology of the symbionts is intimately related to pH (RAYNER **15, 16**), and we realize, as RAYNER states:

> If the investigation can be carried one step farther, it should be possible to offer an explanation of the ultimate causes, not only of the calcifuge habit, but of the edaphic peculiarities of ericaceous plants in general.

MAGROU (**12**), who has recently reviewed the problems of symbiosis and higher plants, states that the "question of the fixation of atmospheric nitrogen by the symbionts has been reopened in connection with the mycorhizas of the Ericaceae."

Summary

1. Soils show striking variations in reaction: (a) For all soil samples tested the range was from pH 2.8 to 8.2, representing a range in "active acidity" from 16,000 to zero, and from zero to 16 in "active alkalinity." (b) The plant associations show ranges in active acidity of considerable extent, the most acid sample in an association being from about 3 to 300 times as acid as the least acid sample. In terms of pH this means a range from 4.3 to 4.8, as in the beech orchard, and from 4.6 to 6.8, as in the chestnut, based on 120 and 63 tests respectively. (c) Variations between three tests per sample

averaged about 0.1 pH from the mean of the three tests, which has increased significance with higher acidities, of course, because of the logarithmic nature of the numbers. Variations ranged from zero divergence of three tests to as much as from pH 4.5 to 5.2. (d) Surface soils tend to show greater variations than subsoils.

2. In respect to the vegetation, certain conclusions as to soil reaction may be drawn: (a) Plant associations show wide ranges of tolerance of hydrogen-ion concentration. (b) No two contiguous associations can be separated on a basis of reaction alone because of the considerable extent of overlapping both in surface and subsoils, although one association may be definitely more acid than another as a general rule (on a basis of averages). (c) Despite this situation it is apparent that the higher acidities particularly exert a considerable influence on the floristic composition of the plant associations. This is probably partly direct (influence of acidity) and partly indirect, because of the influence of peat soils and concomitant factors on the elimination of species.

3. In respect to the general high acidity, certain phenomena are conspicuous: (a) Surface soils are generally more acid than subsoils. (b) Soils of high altitudes are generally more acid than soils of lower altitudes. In a broad way this situation is apparent, but peculiar features of certain associations may enter to upset the perfect altitudinal sequence, as the evergreen habit and peat formation. The altitudinal difference is clearer when we compare identical associations, as chestnut woods at 3000 feet, with an "active acidity" of 55 and at 4700 feet with an "active acidity" of 250, or the heath bald with subsoil acidities of 980 at 5000 feet and 1600 at 6600 feet altitude.

4. Concrete evidence of one type of vegetation being replaced by a more acid-tolerant type, as a result of the tendency of soils in a humid climate to become progressively more acid, with an accompanying altitudinal depression of the limits of zones, has not been obtained as yet but is in all probability a historical fact.

It is a pleasure to acknowledge the interest and assistance of Professor GEORGE D. FULLER, of the University of Chicago, who suggested this field for investigation; and Professor RAY C. FRIES-

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FLORAL MORPHOLOGY OF ARCEUTHOBIUM AMERICANUM[1]

E. Silver Dowding

(with eleven figures)

Introduction

A parasitic phanerogam, *Arceuthobium americanum* Nutt., infects the soft pines in Canada and the United States. The writer first found this plant in 1928, in central Alberta, where it was growing on *Pinus banksiana* and *P. contorta*. It is a yellow plant about 4 inches high, dioecious, and leafless except for the extremely reduced bracts which are opposite and decussate, and which unite to form an inconspicuous rim at the base of each flower. Some observations were first made on the ecology and life history of *Arceuthobium*, and were published in an article on the sandhills of central Alberta (1). It was then found that the morphology of the flower itself was worthy of study, because few botanists have investigated the floral structure, and their findings are not in agreement.

Observations of North American species of *Arceuthobium* have been of a purely ecological nature; it is only on the European *A. oxycedri* (DC.) M.D. that any studies of floral morphology have been made.

In the male flower of *A. oxycedri* the perianth segments vary in number, and it is not known of how many parts the flower originally consisted. The structure of greatest interest in the male flower is the anther, which places *Arceuthobium* in a unique class among angiosperms for two reasons. First, the pollen lies in a continuous ring about a sterile column of tissue. Some observers have stated that the young anther is multilocular at first, while others hold that it is always unilocular. Second, the epidermis of the anther wall takes on the fibrous markings characteristic of the endothecium. Previous investigators seem to be in agreement about this second point. The

[1] The expenses involved in collecting material were defrayed in 1928 by the National Research Council of Canada, and in 1929 by the Royal Society of London.

female flower is known to have two perianth segments, but it is not known how many carpels there are nor how they are arranged. In short, so little is known definitely about the number and arrangement of the floral parts of *A. oxycedri* that there is only meager evidence on which to base any theory of phylogenetic relationship of the genus. This paper is intended to make some contribution along these lines.

The material used for the investigation was *Arceuthobium americanum*, parasitic upon *Pinus banksiana* and *P. contorta*. Two other species were also collected, *A. pusillum* Peck., parasitic on *Picea mariana*, and another species parasitic on *Tsuga heterophylla*, which has previously been known as *A. occidentale* Engelm., but for which ROSENDAHL (6) recommends the name *A. tsugensis*. Examination of available flowers of these two species show that their structure resembled that of *A. americanum* in all important features. For the sake of comparison with a related genus showing a better developed gynoecium, *Comandra pallida* A.DC. and *C. umbellata* (L.) Nutt. were also examined.

Male flower

PREVIOUS INVESTIGATIONS

The male flower is known to consist of a whorl of perianth segments, each bearing a sessile anther. Within the perianth is a central cushion of tissue, which some observers consider to represent the undeveloped gynoecium. Concerning other details of structure of the male flower, there is much difference of opinion.

EICHLER (2) states that the usual number of perianth segments in *A. oxycedri* is three, but that sometimes there are only two. He describes the anthers as bilocular, and opening with a common fissure. JOHNSON'S (4) view of the anther agrees with that of EICHLER, and that of HOOKER whom he quotes. He notes in addition that the epidermis of the anther wall shows the fibrous markings which normally characterize the subepidermal layer. HEINRICHER (3) has frequently found tetramerous male flowers in addition to the trimerous and dimerous ones seen by EICHLER. HEINRICHER states that the anther is unilocular, and describes a central column of sterile tissue which extends to the top of the anther and is surrounded

by sporogenous tissue after the manner of the columella of a moss capsule. STAEDTLER (**8**), having examined the same species, agrees with HEINRICHER that the mature anther is unilocular, but states that it is originally tetrasporangiate because the archesporial tissue is at first divided into quadrants by sterile septa. PISEK (**5**) is not in agreement with STAEDTLER, for he finds from serial sections that the supposed septa in the anther are not complete, and the archesporium is therefore continuous. He regards the septa as projections of a tapetal nature occurring in the central "columella." He confirms JOHNSON's statement that the epidermis becomes the fibrous layer in the development of the sporangium.

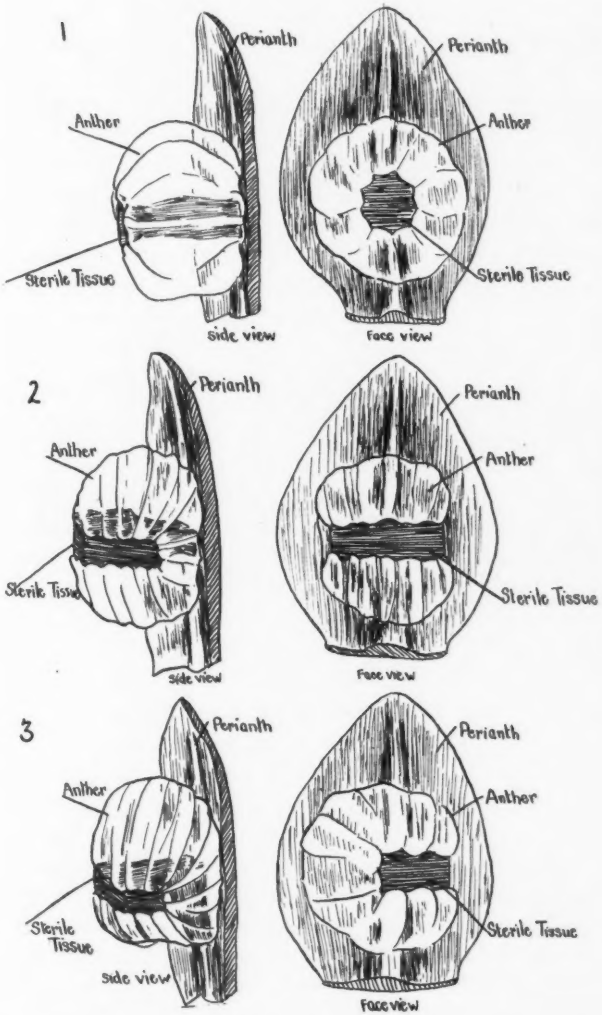
PERIANTH

In *A. americanum* the perianth parts of the male flower are usually three in number, but, as in *A. oxycedri*, there are dimerous, or more frequently tetramerous, flowers. The following facts seem to show that all the male flowers of *Arceuthobium* were originally four partite. (1) A large number of male flowers of *A. tsugensis* were examined and all of them had a perianth of four members. (2) The present investigation shows that the female flower of *Arceuthobium* is tetramerous.

In the dimerous flowers examined, the perianth lobes were always opposite the subtending bracts, the lobes alternating with them apparently having failed to develop. When the flower is trimerous, the arrangement varies; the unpaired segment is usually opposite the subtending bract, but occasionally alternates with it. This variation might be explained by assuming that trimery arose from tetramery by the abortion of one or other of the members.

ANTHER

Examination of a great number of serial sections showed that the anther is unilocular, and penetrated by a sterile column of tissue in the way that the columella penetrates a moss capsule (fig. 1). This has been described by HEINRICHER. Such an arrangement is so unusual that previous workers have studied young anthers to determine whether the archesporium is always a continuous ring from its earliest stages. STAEDTLER reports that the anther is originally multilocular, but that the partitions between the locules break down at



FIGS. 1–3.—Fig. 1, perianth lobes of male flowers of *A. americanum* bearing anthers; common type of anther with "columella" of sterile tissue; fig. 2, anther with sterile tissue forming partition along one plane; fig. 3, showing partitions along two planes.

maturity, leaving the "columella" in the center. PISEK states that the anther is always unilocular, and that the partitions that STAEDTLER describes are merely tapetal strands. My observations support those of PISEK, in that serial sections indicate that the archesporium is continuous in the young anther, but I am not entirely in agreement with him.

After examining a great number of anthers of all ages, I have found that the "columella" exhibits wide variations: it may take the form of a partition dividing the anther almost completely into two sacs (fig. 2); half way up it may die away and a new partition arise at right angles (fig. 3); or the partitions may give out branches which extend to the anther wall. The columella is something more than PISEK's "tapetal strands disappearing at maturity" (5). It persists in the ripe anther; and in spite of variations in position, it can always be distinguished as forming some part or other of the partitions of what apparently was once a tetrasporangial anther. These partitions, tending to divide the single archesporium into four parts, are present in every degree of incompleteness. They are four or five cells thick, only the surface layer having the character of a tapetum. From their permanence, their constancy of position, and their thickness, therefore, it may be concluded that the flanges of the columella are the surviving portions of a tissue which once separated four distinct archesporia.

The perianth of the male flower is discernible in May, and the archesporium early in June. The latter is laid down as a continuous tissue, the sterile portions never being quite complete. This confirms PISEK's observations on *A. oxycedri*, but not those of STAEDTLER on the same species, for he thought he saw four distinct groups of archesporial cells.

In examining the male flower buds in early spring, particular attention was paid to the endothecium, or fibrous layer, of the anther wall, because in the mature anther this layer is outermost instead of being hypodermal, as is the rule in angiosperms. The endothecium, the intermediate layer, and the tapetum are differentiated in early July, and the mode of development of the anther wall is identical with that of *A. oxycedri*, as described by JOHNSON and PISEK. The fibrous layer originates from the outermost layer of the anther wall.

The mature anther in the Ericaceae also has a superficial endothecium, but only because the epidermis which covers the young anther is lost at maturity. To find any families that resemble *Arceuthobium* in the possession of a truly superficial endothecium, we must turn to the Coniferae.

Reduction division of the pollen grain mother cells takes place late in July. Material collected at this time was used for counting the chromosome number. The haploid number for *A. americanum* was found to be 14. (PISEK reports 13 for *A. oxycedri*.)

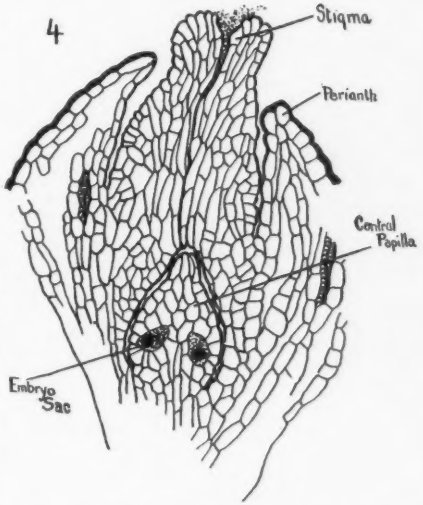


FIG. 4.—Median longitudinal section through young female flower of *A. americanum*, showing position of embryo sacs.

Female flower

PREVIOUS INVESTIGATIONS

The female flower of *Arceuthobium* is described by taxonomists as consisting of a gynoecium and a bipartite perianth, which are fused for most of their length. The style shows a stylar canal which widens to a funnel shape in the region of the stigma (fig. 4). In the main, previous observers of the floral morphology of the female flower are in agreement.

EICHLER (2) represents the perianth segments as standing opposite the inclosing bract, but he does not distinguish carpels. JOHNSON (4) states that in *A. oxycedri* the ovary is made up of two carpels which, like the perianth segments, are also opposite the bract. He observed that in the cavity inclosed by the two carpels there is a central dome of tissue which terminates the axis and bears two embryo sacs in line with the two members of the perianth. HEINRICHER (3) also considers that there are two carpels opposite the perianth leaves.

GYNOECIUM

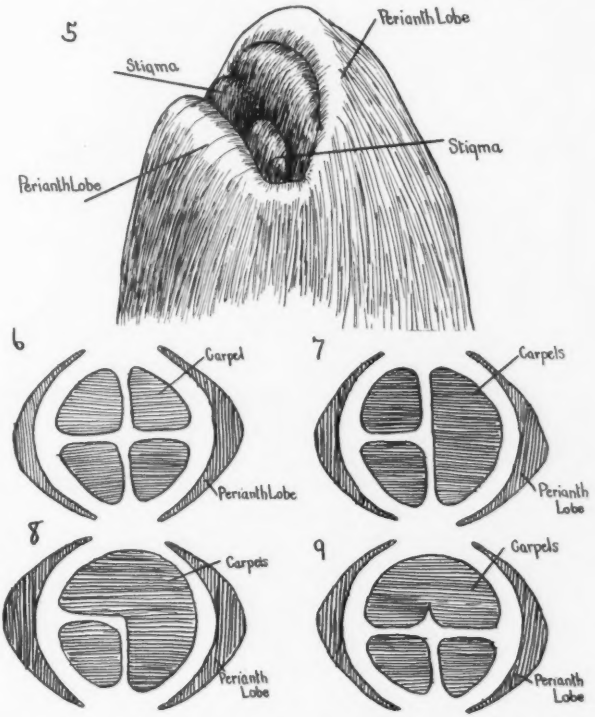
No previous observer has studied the stigma in transverse section. JOHNSON's opinion of the dual number and orthogonal arrangement of the carpels was founded on median longitudinal sections, which, when taken through a funnel-shaped stigma in any of the possible planes, naturally show a right and a left lobe. Although HEINRICHER bases his conclusions of the dual number of carpels on the shape of the stylar canal in transverse section, he gives no illustration of cross-sections of the stigma tip.

Microscopic examination of a dissected stigma showed that it was unequally bilobed (fig. 5). This led to the study of the stigma in transverse section. In the course of the present work, great numbers of transverse sections of the stigma of *A. americanum* were found to show indentations which indicated the presence of more than two carpels. Although the indentations are usually shallow, in some flowers the stigma was evidently divided into four equal parts, the segments being arranged in the diagonal planes (fig. 6). These segments were taken to be four diagonal carpels.

The four carpels fuse in varying degrees. Thus it was observed that any two neighboring carpels may be partially or completely fused (figs. 7, 9), or that the stigma may be bilobed because of three carpels fusing and one remaining free (fig. 8). The usual appearance of the stigma is that of a funnel, the rim of which is unequally bilobed (fig. 5). The larger half, as shown by incomplete fusion, is usually made up of three carpels (fig. 8). In some flowers none of the four carpels is free, and the stigma tip is an entire rim.

EICHLER has already suggested that the carpels might be alternate with the perianth segments, because in the male flowers the central cushion of tissue which probably represents the gynoecium is drawn out into corners between the perianth segments. HEINRICHER doubts that this cushion represents an abortive gynoecium, and points out that its surface shows nothing to indicate nectar secretion; but in *A. americanum* this surface bears numerous pairs of glandular cells not found elsewhere on the axis. In the tetramerous male flower this central tissue might certainly be taken to represent the four diagonal carpels.

The ovules of *Arceuthobium* are imbedded in a central papilla which terminates the floral axis, and which is surrounded by four fused carpels (fig. 4). The central papilla has been previously con-



FIGS. 5–9.—Fig. 5, stigma of female flower showing unequal bilobing; fig. 6, stigma of well nourished terminal flower with four distinct carpels; figs. 7–9, stigmas showing varying degrees of fusion between the four carpels.

sidered as a placenta, that is, the bases of the four surrounding carpels are supposed to project upward to form a central column or placenta to bear the ovules. It is impossible to conceive of the outer carpels having any connection with the ovules. The ovules are situated along the plane where the fused edges of the surrounding carpels lie; that is, the ovules are alternate with the carpels. Also

in *A. tsugensis* and *A. pusillum*, the two ovules were found in the orthogonal plane and the four carpels in the diagonal planes. This arrangement leads to the following important deduction: the four outer carpels are sterile and bear only stigmas, while the embryo sacs are borne on an inner whorl of orthogonally arranged carpel rudiments.

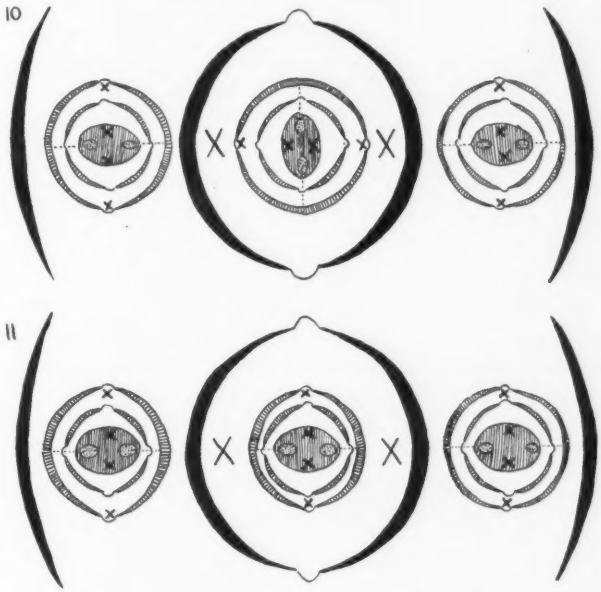
In the middle of July the archesporial groups can be seen in the central papilla. At no time are they in the form of single cells, as JOHNSON thinks for *A. oxycedri*, for each group is made up of as many as ten cells. The number of archesporial groups in the central cone appears to be four, and not two as was previously supposed, and they are arranged so that they alternate with the sterile carpels. The gynoecium might then be represented as G=4+4.

Although the archesporia in the lateral plane, that is, those which are to give rise to the two embryo sacs, are as a rule much more strongly developed than the two in the median plane, some evidence that the other pair of embryo sacs may sometimes develop at the expense of the lateral pair has been found in a single terminal flower, which will now be described.

Usually the inflorescence terminates in a cluster of three flowers, and these are oriented so that the planes of symmetry through the embryo sacs are parallel, and, at the same time, are all at right angles to the planes of symmetry through the embryo sacs of the two lateral flowers at the node below. When the lateral flowers of the terminal cluster do not develop, so that the terminal flower is solitary and surrounded by two empty bracts, the plane of symmetry through the embryo sacs of the terminal flower is still at right angles to that of the laterals on the node below (fig. 10). One solitary terminal flower was found, however, which had its plane of symmetry through the embryo sacs parallel to the laterals of the node below (fig. 11). This change in the plane of symmetry of the terminal flower to a plane at right angles to the usual one (cf. figs. 10 and 11) indicates that perianth segments may develop which alternate with those usually found, and also that alternate embryo sacs may develop at the expense of those ordinarily encountered.

The development of the alternate pair of embryo sacs might be expected, since it has been shown that in *A. americanum* four archesporia are laid down. A similar alternate development for the

perianth is possible because the two segments that normally appear do not occupy the whole circumference of the perianth tube (fig. 5). (In *Viscum* the perianth is four partite.)



FIGS. 10, 11.—Floral diagrams of terminal clusters of female flowers (composed of three whorls, outer, the perianth and inner two, the gynoecium; bracts shown black; *x*, aborted flowers or aborted members of floral whorl); fig. 10, usual arrangement of terminal flower in relation to laterals of node below; fig. 11, unusual arrangement of terminal flower in relation to laterals of node below, indicating that alternate pair of embryo sacs has developed in terminal flower in place of usual ones. Planes of symmetry shown by dotted lines.

COMPARISON WITH GYNOECIUM OF COMANDRA

Strong evidence that there is an alternation of sterile and fertile carpels in a number of families of flowering plants has been brought forward by SAUNDERS (7). Additional evidence supporting the carpellary polymorphism of *Arceuthobium* has been sought for in the Santalaceae, because in this related family reduction due to parasitism has not gone so far.

As compared with the evascular nature of the ovary of *Arceutho-*

bium, in the gynoecium of *Comandra* the ovary wall has a well marked vascular system. The ovarian papilla of *Arceuthobium* is replaced by a spirally twisted "placenta" bearing pendulous ovules and containing a vascular strand. The number of the members making up the ovary cannot be determined by any indentation of the stigma, as this is entire, nor can any help be obtained from a determination of the position of the embryo sacs, as these are borne on a twisted column. There is only the vascular system on which to depend.

In *C. umbellata* and *C. pallida* the perianth tube is traversed by five main vascular bundles, which run to the five sepals and their superposed stamens. Five vascular strands branch off from these bundles and, taking up a position alternate with the main bundles, run for a short distance into the base of the carpels. Farther down in the peduncle five other vascular strands arise, which are alternate to the five bundles which run to the base of the carpels. After anastomosing, they pass into the central column bearing the ovules. The whorl of vascular tissue which supplies the "placenta" is alternate to the one which supplies the outer carpels. This indicates that the gynoecium is composed of two alternate whorls of carpels, rather than one whorl bearing a placenta, as previously supposed.

Formation of embryo sac

The ovules of *Arceuthobium* are without integuments, and consist simply of embryo sacs imbedded in the central papilla (fig. 4). According to Johnson, in the development of the archesporium in the female flower of *Arceuthobium* a number of tapetal cells are cut off. Not the slightest evidence was found to support this view. The embryo sac mother cell divides to form two daughter cells, the upper of which is the embryo sac. In some flowers the lower daughter cell is much smaller and soon disintegrates. The embryo sac then develops normally. In other flowers the upper cell and lower cell are equal, and for some weeks keep pace with each other in further development, the nuclei dividing simultaneously. The embryo sac eventually enlarges at the expense of the lower cell and forms seven nuclei in the usual way.

Summary

MALE FLOWER

1. The male flower is thought to be originally tetramerous.

2. The anther, although unilocular, is found to be characterized by a system of sterile tissue which represents what is left of the separating partitions of a tetrasporangiate anther.

3. The haploid chromosome number is 14.

FEMALE FLOWER

4. The ovary wall is made up, not of two fertile but of four sterile carpels. These are diagonally arranged and show varying degrees of fusion.

5. The two embryo sacs in the central papilla lie in the orthogonal plane, and therefore must be borne on an inner alternating whorl of orthogonally arranged carpel rudiments. This inner whorl bears not two but four archesporia, only one pair forming embryo sacs. The structure of the gynoecium must therefore be represented as $G=4+4$ and not as $G=2$.

6. The archesporia are not unicellular but multicellular.

7. The gynoecium of the one genus of the Santalaceae examined resembled *Arceuthobium* in being composed of two alternating whorls of carpels.

The work recorded in this paper was carried out at Cambridge University during the tenure of a Traveling Scholarship awarded by the Canadian Federation of University Women, and the manuscript was completed at the University of Manitoba. I have much pleasure in expressing indebtedness to Miss EDITH R. SAUNDERS of Newnham College, for valuable help and criticism during the progress of the work.

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A GROUP OF TETRAPLOID ROSES IN CENTRAL OREGON[1]

EILEEN WHITEHEAD ERLANSON

(WITH THREE FIGURES)

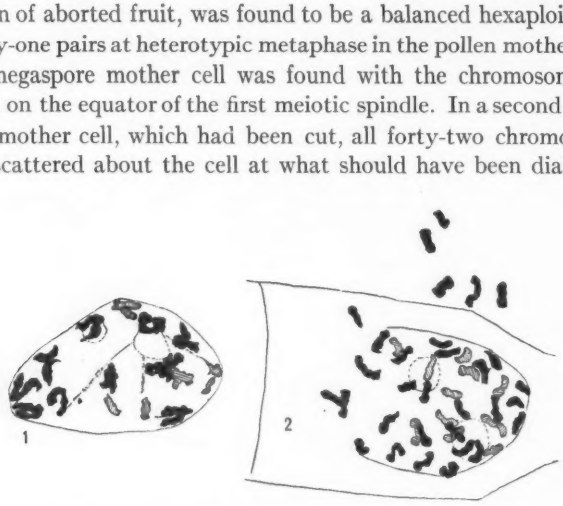
In the course of a western collecting trip in 1928, the writer was taken by Dr. H. M. GILKEY to a large colony of interesting wild roses near Corvallis, Oregon. Hedgerows along either side of a lane consisted of *R. pisocarpa* A. Gray (diploid) and related forms, also *R. nutkana* Presl (hexaploid) and some related forms, and the rare species *R. durandii* Crépin, which is apparently confined to the Willamette River region (ERLANSON 3). Two large bushes were also observed on one of which all the hypanthi had aborted, the other showing about 50 per cent of aborted fruit. These were judged to be hybrids between one of the hexaploid forms present and *R. pisocarpa*.

In the spring of 1929, young buds from four individuals in this colony (including *R. durandii*) were kindly fixed by Dr. GILKEY in acetic alcohol and forwarded to Michigan. Stages in reduction division were found in each collection. Herbarium vouchers also were collected by Dr. GILKEY from the bushes which provided the cytological material. All these bushes resemble *R. nutkana* in habit and general morphological characteristics, including the inflorescence of large flowers, borne either singly or two to three together on short lateral branches. This type of inflorescence I had heretofore found always associated with the hexaploid condition in American roses. *R. durandii* is unique among the wild roses on this continent in possessing puberulent branches and large compressed alate prickles, which are also puberulent. It has been regarded by several students of the genus as related to *R. nutkana* (RYDBERG 12). It was a distinct surprise, therefore, to find that three of the collections sent by Dr.

[1] Papers from the Department of Botany of the University of Michigan no. 324, representing work carried on under a National Research Fellowship in the Biological Sciences.

GILKEY, including *R. durandii*, were tetraploids with twenty-eight somatic chromosomes and fourteen pairs at diakinesis.

One of the plants, which in the autumn of 1928 bore a large proportion of aborted fruit, was found to be a balanced hexaploid with twenty-one pairs at heterotypic metaphase in the pollen mother cells. One megaspore mother cell was found with the chromosomes all paired on the equator of the first meiotic spindle. In a second megaspore mother cell, which had been cut, all forty-two chromosomes were scattered about the cell at what should have been diakinesis



FIGS. 1, 2.—Fig. 1, diakinesis in megaspore mother cell of *R. durandii;* 13 pairs and 2 univalent chromosomes. Fig. 2, megaspore mother cell of *R. nutkana* var. from Oregon: diakinesis, no pairing, 42 univalent chromosomes; cell cut, 5 chromosomes in adjoining section shown at side of main part of cell; ×2000.

(fig. 1). These roses are distinctly protandrous, in that the megaspore mother cells are entering reduction division just before the bud is ready to open, and the anthers contain mature pollen. Since it is difficult to find many megaspore mother cells undergoing meiosis, fig. 1 is presented as an interesting observation, although no deductions can be made as to its significance in relation to the partial ovule sterility of the bush until more stages in embryo sac production have been seen. Such a nucleus might give rise to a diploid megaspore or to an apogamous embryo. Pollen taken from the herbarium specimen sent with the cytological material showed only 12 per cent of empty shriveled grains after treatment with aceto-carmine. The writer has examined pollen samples from numerous hexaploid roses, both in the group of *R. acicularis* Lindl. and of

R. nutkana, and has found less than 15 per cent of empty and dwarf grains in all plants but one, and usually less than 10 per cent.

Reduction division in the three tetraploid specimens is almost always regular, with infrequent cases of lagging of one or two pairs at first metaphase and of failure to pair on the part of two of the chromosomes. Fig. 2 shows diakinesis in a megaspore mother cell in *R. durandii*. Groups of four chromosomes in a ring are rare, and when present one group only has been observed in a cell. These are the same irregularities as those found in *R. californica* and in eastern tetraploid species (**2**, figs. 38, 39, 49).

Pollen taken from Dr. GILKEY's herbarium specimens showed that the *R. durandii* bush had 14.8 per cent sterile pollen. A second tetraploid had 16.6 per cent, and the third 54.6 per cent empty grains; these were not dwarf grains such as result from polyspory but full sized spores whose content had disintegrated after tetrad formation. In some anthers whole blocks of pollen grains had no protoplasmic contents. This type of pollen sterility, which is not associated with unusual meiotic irregularity, resembles that found in the sterile diploid type of *Primula kewensis* (DIGBY **1**, NEWTON and PELLEW **8**). In the pollen sample exhibiting 54.6 per cent sterile grains, one giant grain twice the volume of a normal grain, with protoplasmic contents, was seen. Such grains are not infrequent in rose pollen exhibiting a high sterility. They indicate the occasional failure of reduction in some spore mother cells, which have probably undergone what ROSENBERG (**9**, **10**) designated as a "hemiheterotypic division."

TAXONOMIC POSITION OF FORMS.—The hexaploid specimen is a vigorous, much branched bush over 5 feet high. The vouchers sent by Dr. GILKEY fit the descriptions of *R. nutkana* given by RYDBERG (**11**), except that the branches are completely unarmed.[2]

The tetraploid specimens are *R. durandii* and two related forms, both of which possess the solitary flowers, glandular hispid pedicels, and leaflets (both glandular and puberulent beneath) of that species. They differ in having glabrous stems and prickles. One speci-

[2] RYDBERG suggested (Bull. Torr. Bot. Club **44**:69. 1917) that the unarmed specimen from the Aleutian Islands designated as *R. aleutensis* by CRÉPIN (1875) may have been an unarmed form of *R. nutkana*.

men has straight stout prickles and might be a tall form of the dwarf *R. yainacensis* Greene. The plant with semisterile pollen has small curved prickles and the branches are sparingly glandular-hispid at the tips; it might be a stout form of *R. myriadenia* Greene which is known only from the type locality in Jackson County, Oregon. These tetraploid plants are not so tall nor so much branched as is typical *R. nutkana*, and in the field recalled some of the Gallicae (ERLANSON 3). Plants similar to them in habit were also collected at Crescent City and Fort Bragg, California, in 1928, and were determined as *R. brownii* Rydberg and *R. muriculata* Greene. The Botanical Garden of the University of Michigan has a hexaploid specimen of *R. muriculata* (no. 2931, Seattle, Washington, legit T. C. Frye), with graceful branching stems 6 feet high.

We have, therefore, a group of tetraploid roses of somewhat local distribution in central and southern Oregon, probably extending into northern California. They resemble the hexaploid roses in the group of *R. nutkana*, and the distinguishing morphological characteristics which they possess are of doubtful taxonomic value. Prickle shape is highly variable in nearly all rose species. All the polyploid roses of the Pacific Coast region of the United States have stout compressed prickles; in the group of *R. nutkana* they are usually straight, and in the group of *R. californica* C. & S. usually curved. Specimens of *R. nutkana* with pubescent stems have not been reported. This characteristic was observed by me in an apparently diploid specimen in Utah and in *R. ultramontana* S. Wats. in northwestern Nevada. Specimens belonging to the group of *R. californica* from San Luis Obispo, collected by *Mrs. R. W. Summers* (nos. 256 and 258, 1903), are in the herbarium of the University of California; they have the tips of the branches and pedicels often puberulent or finely glandular. Other specimens related to *R. californica* have been seen with the tips of the branches glandular hispid. This character is therefore not restricted to *R. durandii* alone on this continent.

In order to discover whether there were any other morphological characteristics by which these tetraploid roses could be distinguished, the stamens and the teeth on the leaflets were counted. Although the serrations along each side of the leaflets are scarcely more numerous than in the Oregon hexaploid specimen (table I),

they are distinctly finer and more like those of *R. pisocarpa* (fig. 3). The number of stamens in the four Oregon specimens is given in table I. The hexaploid has 76–85 and the three tetraploids 110–130.

TABLE I

SOME QUANTITATIVE CHARACTERS DISTINGUISHING FOUR OREGON ROSES

CHARACTERS	R. NUTKANA VAR.	R. DURANDII	R. MYRIADENIA VAR.	R. VAINACENSIS VAR.
Stamens in two flowers	76 85	110 112	115 130	113 124
Sterile pollen	12%	14.8%	54.6%	16.6%
Size of round pollen grains	26.6–30 μ	25–26.6 μ	23.3–26.6 μ	
No. of teeth on side of leaflets	9–14	10–17	10–17	9–15
Mean number of teeth	10.2	12.9	13.2	10.7

Since the counts were made on dried specimens they are likely to err on the low side. Counts of stamens made on living plants have shown that *R. nutkana* has 90–110 and *R. californica* 105–130 stamens.

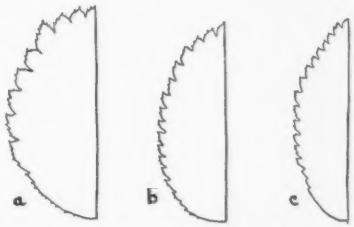


FIG. 3.—Serrations of leaflets in rose specimens from Corvallis, Oregon: (*a*) *R. nutkana* var., (*b*) *R. durandii*, (*c*) *R. myriadenia*; ×2.

Studies on rose pollen have shown that the size of the microspores increases with the increase in the chromosome number, as in wheat (SAX 13) and other polyploid genera. In table II are listed the sizes of grains found in rose pollen examined in samples from 400 individuals of the section Cinnamomeae in the Botanical Garden of the University of Michigan. Only those grains which appeared to be perfect morphologically were measured. There is some overlapping between the sizes of the spores in the three cytological groups diploid, tetraploid, and hexaploid. The variation in pollen grain size shown for each of these groups is found in almost all samples, so that the average size for any individual in the same group will be about

equal. When rose microspores reach maturity they are shaped like grains of wheat, with rounded ends, in which condition they are usually shed from the anthers; later they become spherical. The size differences between pollen from plants with unlike chromosome numbers (table II) are more easily observed when the spores are oval in shape.

TABLE II

SIZES OF POLLEN GRAINS IN DIPLOID, TETRAPLOID, AND HEXAPLOID ROSES

CYTOLOGICAL TYPE	LENGTH OF OVAL GRAINS (μ)	DIAMETER OF ROUNDED GRAINS (μ)
Diploid	30–39.4	20–25
Tetraploid	38.3–46.6	23.3–26.6
Hexaploid	45–51.6	25–30*

* Very few samples of hexaploid pollen have been seen in which the grains have rounded off.

Three of the Corvallis specimens showed only rounded grains. The spores of *R. durandii* and of *R. myriadenia* var. were distinctly smaller than those of the *R. nutkana* specimen (table I). Oval spores were obtained from the fourth specimen which were 40–43.3 μ long, which would class them as tetraploid without much doubt. In *R. californica* (tetraploid) oval pollen grains vary from 40 to 46 μ in length.

In the light of the cytological findings in the Corvallis roses and of field and herbarium studies, I am led to suspect that the following rose species are tetraploids:

OREGON	NORTHERN CALIFORNIA
R. durandii Crépin	R. brownii Rydberg
R. myriadenia Greene	"R. muriculata" (in southern part of its range)
R. yainacensis Greene	R. calavera Greene
R. delitescens Greene	R. pinetorum Heller

Measurements are given in table III which were made on the pollen taken from three herbarium specimens not collected by the writer. The specimen collected on Mount Eddy by HELLER, and determined by him as *R. delitescens*, does not agree with GREENE'S description. It is clearly a hexaploid judging from the size of the

microspores. It also has the low percentage of sterile grains characteristic of American hexaploids. It is probably a glabrous and eglandular form of *R. nutkana*. The specimen of *R. brownii* might be either tetraploid or hexaploid by the size of the rounded pollen grains. The high percentage of sterile grains suggests that it is more likely to be tetraploid.

TABLE III

POLLEN MEASUREMENTS FROM HERBARIUM MATERIAL OF WESTERN ROSES

SPECIES* AND LOCALITY	SHAPE OF GRAINS	SIZE IN μ (DIAMETER OR LENGTH)	PERCENTAGE STERILE GRAINS	INFERRED CYTOLOGICAL CLASS
R. delitescens, Mt. Eddy, Calif. Leg. A. A. Heller, no. 13254, June 20, 1919	Oval	45–51.6	11.6	Hexaploid
Same, no. 13507	Oval	45–50	.....	Hexaploid
R. brownii, Trinidad, Humboldt Co., Calif., Leg. H. H. Smith, no. 3821, June 7, 1911	Round	23.3–26.6	37.5	Tetraploid
R. pinetorum, Pacific Grove, Calif., Leg. A. A. Heller, June 3, 1903	Round	23.3–25	55.6	Tetraploid
R. yainacensis, Stirling, Butte Co., Calif., Leg. A. A. Heller, no. 10809, June 7, 1913	Round	23.3–25	41.3	Tetraploid

* The species were determined by the writer except for the first two specimens.

POSSIBLE ORIGIN OF NEWLY FOUND TETRAPLOID GROUP

Following HURST'S theory (**6, 7**), the discovery of tetraploid forms resembling *R. nutkana* and growing near the southern limit of its range, one might suppose that they had been derived from it by the loss of one double septet (fourteen chromosomes). Such a supposition is not in accord with the cytological behavior of polyploid species in genera which provide good material for experimental work. That the higher polyploids in *Rosa* were originally built up by the processes of hybridization and chromosome duplication is admitted by HURST (**7**), and it is more in line with our present knowledge to postulate that the chromosome number may be reduced by the intervention of hybridization between species having different chromosome numbers. An alternative explanation, therefore, is to suppose

that the hexaploid *R. nutkana* and the tetraploid *R. californica* have hybridized in the extreme southern region of Oregon, where their ranges overlap. Such a crossing would give an unbalanced pentaploid in F_1 with possibly fourteen pairs and seven unpaired chromosomes at reduction division. Many of the F_1 gametes would probably receive only fourteen chomosomes, because the unpaired chromosomes usually lag during the anaphases of meiosis, and an almost fertile tetraploid race might be produced strongly resembling one of the original parent species but exhibiting some characters of each parent.

Examples are known in other polyploid genera in which interspecific hybridization gives rise to offspring resembling only one of the parent species. In a cross between *Nicotiana tabacum* and *N. sylvestris*, GOODSPEED and CLAUSEN (**4**) obtained F_1 plants which resembled the *tabacum* parent entirely. When these were backcrossed to the *sylvestris* parent, the resulting offspring resembled *N. sylvestris* (**5**). Plants from a cross between *Triticum dicoccum* ($x = 14$) and *T. vulgare* ($x = 21$) yielded almost all *dicoccum*-like individuals in F_2, in the experiments of THOMPSON and HOLLINGSHEAD (**14**). They also found that the majority of the F_2 plants showed fourteen bivalent chromosomes and only 0–4 univalents at meiosis. These investigators attribute this to the lagging of univalent chromosomes in the F_1.

Another crossing that might be considered is one between *R. nutkana* and a diploid of the *R. pisocarpa* group, followed by autosynezesis among the fourteen unmated *R. nutkana* chromosomes at first metaphase in the F_1. One would not then expect to find such strong, variously curved prickles, such a preponderance of glandular foliage, such pubescent branches, nor such an increase in the number of stamens, in the descendants of such a cross. *R. pisocarpa* has fewer stamens than even *R. nutkana;* at least this is true for two plants from Oregon which have been able to persist in Michigan.

These tetraploids are unique among American tetraploid roses yet observed by the writer in not seeming to produce flowers on the annual shoots or turions, and in having a simple inflorescence of from one to three (rarely four) flowers, as in our hexaploid roses (ERLANSON **2**). The ability to flower on turions is best developed

in the group of *R. arkansana*, and is only occasionally seen in *R. californica* and its relatives.

Summary

1. Cytological material from Corvallis, Oregon, of *Rosa durandii*, *R. myriadenia*, and *R. yainacensis* showed that these are balanced tetraploids with fourteen pairs at diakinesis. A semisterile *R. nutkana* was hexaploid.

2. These tetraploid roses resemble *R. nutkana* in the size of flowers and hips, and in inflorescence type. In the number of stamens, fine serrations of the leaflets, size of pollen grains, and amount of sterile pollen they resemble the tetraploid *R. californica*.

3. This tetraploid group seems to have a limited range, perhaps from central Oregon to the northern counties of California. It may have originated from a crossing between *R. nutkana* and *R. californica*.

4. It is suspected that some other rose species of limited range in this area belong to this group. They are *R. delitescens*, *R. brownii*, *R. calavera*, *R. pinetorum*, and the forms in northern California which are usually classified as *R. muriculata*.

5. It is suggested that pollen grain size might be used in distinguishing the tetraploid and hexaploid rose forms in herbaria. The cubes of the mean length of the pollen of diploid, tetraploid, and hexaploid are as 1 : 1.7 : 1.9.

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MERISTEM IN OSMUNDA CINNAMOMEA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 411

GEORGE L. CROSS

(WITH FIFTEEN FIGURES)

Introduction

The stele of the Osmundaceae has interested morphologists and anatomists for the past half century. DE BARY (9) first called attention to the fact that *Osmunda* in many respects exhibits a certain similarity to the type of cauline vascular system found in the majority of the seed plants. FAULL (10) described the adult anatomy of the group but did not attack the problem from the standpoint of the origin of tissues. JEFFREY (12) prepared a monograph in defense of his theory of the cortical origin of pith, using living and fossil forms of *Osmunda* to illustrate a series of advancement, beginning with *O. skidegatensis* (Jurassic) and ending with *O. claytoniana*. VAN TIEGHEM used practically the same series equally well in defense of his stelar theory. Despite the variation in interpretations of different investigators, however, it is generally conceded that the stele of *Osmunda* is very different from that of most other pteridophytes.

The present study was undertaken with the idea that a detailed investigation of the growing tips of the three prominent living species of *Osmunda* might throw light on the anatomical situation of the family as a whole. Although this paper is concerned almost entirely with *O. cinnamomea*, it is planned to make subsequent comparative studies of *O. claytoniana* and *O. regalis* before drawing conclusions relative to the phylogenetic anatomical situation of any of the species.

Material and methods

The material for the investigation was collected by the writer in the valley of the Calumet River, near Chicago.

Sections of the rhizomes were prepared for study by the paraffin method exclusively. To aid in cutting, the mass of sclerenchyma invariably found in the pith of *Osmunda cinnamomea* was dissected

out by making a longitudinal slit on one side of the stem through the cortex and stele when it was desired to cut sections any appreciable distance from the apex of the stem.

Much trouble was experienced in selecting appropriate killing and fixing reagents. Formalin-acetic-alcohol in any of the proportions recommended by CHAMBERLAIN[1] caused plasmolysis and rupture of the cells at the apices of root, stem, and leaf. Formalin-alcohol alone gave scarcely better results. Corrosive sublimate, hot or cold, occasionally gave good fixing, but as a rule failed entirely. Chromo-acetic acid penetrated very poorly and in many instances caused maceration of the material. Perhaps the most satisfactory killing and fixing reagent employed was 10 per cent neutral formalin, although some destruction of the large apical cells occurred by this method.

General anatomy

The rhizome is a massive, semisubterranean, slow growing structure, having heavy leaf bases and an outer cortex impregnated with sclerenchyma. The stem branches, probably dichotomously. The leaves arise at the apex, and in acropetal succession form a close spiral, the phyllotaxy of which has been worked out by FAULL (11). The leaf traces are concentric and endarch. The stem, to use JEFFREY'S nomenclature, is a phyllosiphonic mesarch siphonostele, varying from the amphiphloic condition (*O. skidegatensis* and sometimes *O. cinnamomea*) to the ectophloic (*O. regalis* and *O. claytoniana*). Maturation at the tip of the rhizome proceeds slowly, and in consequence all tissues mature at a relatively great distance from the apex, although the xylem and protophloem are differentiated early. The roots arise opposite the wings of metaxylem, two for each leaf trace in *O. regalis* and *O. claytoniana*, and but one in *O. cinnamomea*.

STEM TIP

An apical cell or a group of horizontally seriated initials is always present. The first major work on the stem apices of the Osmundaceae was done by BOWER (2), who investigated *O. regalis* in detail. He describes the growing tip and reports finding a four-sided initial cell in a few apices. He states that there is present in most forms a

[1] Methods in plant histology. Chicago. 1915.

conical apical cell which is rather deep in proportion to its height, but he concludes that the apical meristem on the whole is of a "less regular and definite type than in the leptosporangiate ferns."

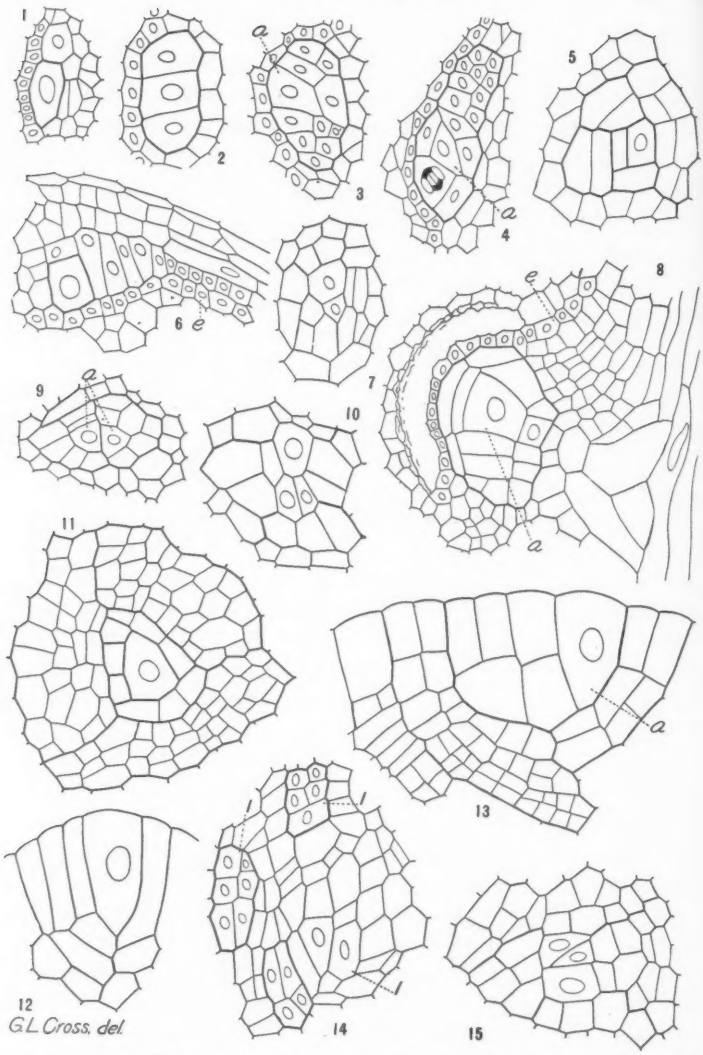
My investigations were concerned almost exclusively with *O. cinnamomea*, although a sufficient number of preparations of *O. regalis* were made to verify the work of BOWER. In general topography, the apex of *O. cinnamomea* is much the same as the apex of *O. regalis*. The cells are relatively large, thin walled, and watery.

The apices of a great number of rhizomes were sectioned. Plants of all ages and sizes were selected, from the sporeling stage up to extremely old rhizomes. Both longitudinal and transverse sections were prepared.

In the sporeling, as noted by CAMPBELL (7), a triangular pyramidal apical cell is prevailingly present. This cell persists during the youth of the rhizome, and frequently may be found in middle aged plants. The relative size and shape of the cell are shown in fig. 11. Obviously the segmentation does not proceed with the regularity characteristic of the typical leptosporangiate fern. The segments are not cut off in spiral succession as a rule, although this mode of growth sometimes occurs intermittently for a small number of segments (4–6). The identity of the segments is soon lost, due to the relatively great length of time elapsing between successive segmentations, and to the numerous divisions occurring in the segments themselves.

Before the young rhizome forks, the apical cell apparently divides into two equal portions, in each of which is organized a new apical cell that functions as the growing point of the divergence. Fig. 9 was drawn from the growing tip of a young rhizome, which was about to fork in the dichotomous manner described.

As the rhizome ages, segmentation at the apex becomes progressively less regular, and differential growth, or twisting of the stem tip due to growth of the leaves, may pull the apical cell into such position that it appears to be four-sided (fig. 14). As the segmentation becomes more and more irregular, the identity of the original triangular pyramidal apical cell becomes lost, and it is replaced by a group of initials which function as a meristem (fig. 10). The old rhizomes may branch, but infrequently. FAULL records *Osmunda* as



FIGS. 1–15.*—Fig. 1, young root initial arising in pericycle just within endodermis (at left); cross-section of rhizome. Fig. 2, young root in 3-celled stage with endodermis

* All figures drawn at level of table with a Spencer camera lucida, under a 4 mm. Leitz apochromatic objective, with periplan ocular 8×; reduced to one-fourth original size.

branching once. I have observed several cases in which a single rhizome branched three times. After branching, the older portions of the rhizome decay with the advent of time and two individuals result. Such a method of reproduction is characteristic of practically all plants with an underground branching stem. Fig. 13 is a representative longitudinal view of the apex of a rhizome which is on the verge of losing its growth by means of an apical cell, in favor of growth by meristem of two or three cells. To the left of this figure is shown the origin of desmogen tissue and a possible leaf initial. The sequence of segments may be traced here to better advantage than in any other rhizome studied. It can be seen that the segmentation has been regular, and almost of the leptosporangiate type until the last three or four segments, when the apical cell was apparently thrust to one side by the overgrowth of one of the lateral derivatives. This lateral derivative then, instead of undergoing the usual periclinal division, divided in a plane perpendicular to the surface of the apex, resulting in the formation of two cells approximately equal in rank to the apical cell. Extensive studies of like situations would indicate that this is a preliminary step in the replacement of the apical cell with a meristematic group. The other

to left; cross-section of rhizome. Fig. 3, longitudinal section of young root with definite apical cell (*a*); segments which will contribute to formation of root cap not yet formed. Fig. 4, longitudinal section of young root with definite apical cell which has cut off initial cell of root cap to left; endodermis at left. Fig. 5, cross-section of young root passing through cortex of rhizome; sequence of segments obviously irregular. Fig. 6, longitudinal section of rhizome showing enlarged root initial being formed in pericycle just within endodermis (*e*). Fig. 7, cross-section of young root entering soil; individuality of original apical cell is being lost, and a 2- or 3-celled meristem apparently taking its place. Fig. 8, longitudinal section of rhizome and young root showing truncate appearance of 5-sided apical cell, and portion of leaf trace with which vascular system of root will ultimately become continuous. Fig. 9, cross-section of apex of young rhizome just before forking, showing apical cells of the two branches. Fig. 10, cross-section of apex of old rhizome with apparently 3-celled meristem. Fig. 11, cross-section of apex of very young rhizome showing definite tetrahedral apical cell which has undergone rather definite segmentation; leaf initials not distinguishable. Fig. 12, longitudinal section of apex of young rhizome with definite tetrahedral apical cell. Fig. 13, longitudinal section of old rhizome; growth slow and individuality of original apical cell practically lost; elongated cells which later become desmogen tissue at left. Fig. 14, cross-section of apex of old rhizome; irregular triangular apical cell represented with three young leaves (*l*) and possible initial of fourth. Fig. 15, cross-section through apex of old rhizome showing one of many irregular types of meristems found in this species.

possibility remains, however, that this is merely an expression of one of the many irregularities that may be expected in the apical meristem of any of the Osmundaceae, even if a functioning apical cell be present.

Whatever the type of segmentation, lateral derivatives of the apical or master cells divide, at first periclinally, and then in three planes in such a manner as to form two or four large outer cells and a number of smaller inner cells for each segment (fig. 13). The latter elements form a solid dome-shaped structure, the peripheral members of which give rise to desmogen tissue, unbroken by leaf gaps. The larger outer cells mentioned in connection with the lateral derivatives of the apical cell undergo a few anticlinal divisions, and ultimately certain of their progeny give rise to leaf initials, while the remainder contribute to the undifferentiated epidermis of the leaf.

Root origin and development

An effort was made to determine the histological relationships of the tissue in which the root initial appears, but, due to the slow differentiation and maturation of the stem, combined with the early origin of leaf traces, decisive information was not obtained. A number of factors indicate that the young root arises in a tissue which is on the border line between the stele and cortex, and which later becomes differentiated into pericycle (figs. 1, 6). Studies in this connection indicate that this tissue may not be traced to any definite cell derived from segments cut off by the apical cell or meristematic group.

The initial cell of the root may be observed very near the tip of the rhizome. It is conspicuous on account of its large size and by its nucleus, which is usually nearly twice as large as the nuclei of the surrounding cells (fig. 1).

As in the case of the stem, development of the root is characterized by extreme irregularity. Two, or even three initial cells may be involved in the origin of the root, one of which may give rise to an apical cell, or the group may form an irregular meristem. After examination of a great number of rhizomes, the conclusion was reached that the mechanics involved in probably more than half of the cases are in accordance with the following description: Shortly

after the root initial is differentiated, it undergoes two divisions in a plane parallel, or nearly parallel to the longitudinal axis of the rhizome and perpendicular to its surface. Due to the growth of the leaf trace, with which the vascular system of the root will ultimately become continuous, the resulting cells may be slightly stretched in a plane oblique to the axis of the rhizome, a fact which makes it extremely difficult to obtain median sections, regardless of how the rhizome be oriented in cutting. The three cells obtained by the two divisions just described often undergo a period of enlargement before further divisions occur (fig. 2). The surrounding tissue continues to divide, thus keeping pace with the enlargement of the root initial and the growth of the leaf trace with which the vascular system of developing root becomes continuous.

In *Osmunda cinnamomea* the prevailing situation is one root for each leaf, but a few instances were observed in which two root initials had been formed for a single leaf; one of them, however, proceeded only to the formation of a few cells before its growth was inhibited.

After the three-celled stage the young root develops rapidly. An oblique wall is laid down in the central cell, resulting in the formation of a wedge-shaped element which functions as the apical cell of the young root. The lateral members of the original three also divide into a number of more or less rectangular cells (fig. 3). Sometimes one of these lateral cells may divide in a manner suggestive of an apical cell (fig. 4), but its activities are soon altered and subordinated if the original apical cell is to retain its identity. If these lateral cells divide and keep pace with the apical cell, a multicellular meristem results.

The apical cell of the young root just described has five cutting faces. It may be triangular in profile, or truncate at the base (fig. 8). The four posterior faces cut off segments which form the body of the root, but contribute also to the root cap, while the fifth anterior face contributes to the formation of the root cap exclusively. Fig. 8 was drawn from a longitudinal section of a young root. The apex of the root is capped by a layer of cells which are probably endodermal, but have not been derived from the apical cell of the root. Such a situation is frequently found in angiosperms, where the lateral roots

are distinctly pericyclic in origin. The layer of cells, which may be the undifferentiated endodermis, is easily distinguished at the time the root initial is set off from the rest of the tissue (figs. 1, 6). Its histogenetic relationship is not clear, as already indicated, due to the frequent interruptions of the continuity of the stele by leaf gaps. It should be noted, however, that at the time of the origin of the root initial the pericycle consists of but one layer of cells (fig. 6), and is distinct from the endodermis. Tracing such sections upward toward the apex of the rhizome has resulted in strong but not positive evidence for the independent origin of the pericycle and endodermis.

A few cases were observed in which a triangular pyramidal apical cell might have been present. The same condition has been reported for the older roots of *Osmunda regalis* by BOWER (3). In none of the cases, however, did the triangular cell function with the diagrammatic regularity so characteristic of the leptosporangiate ferns. Such apices are regarded as possessing a meristematic growing tip (fig. 7) rather than a triangular apical cell. The majority of the young roots had a five-faced apical cell, as previously described.

Segmentation may be counter-clockwise for a time (three or four segments), but as the root enlarges and starts to pass through the cortex of the rhizome, the walls seldom follow in the typical spiral succession. Fig. 5 represents a cross-section of such a root. The irregularity of placement of walls may readily be observed, and deemed doubly significant, inasmuch as, of the fifteen or twenty cases examined, this root was selected as having the most regular segmentation. Irregularity increases with age, and in old roots the single apical cell gives rise, in many instances, to a pair, three, or even a group of initials, horizontally seriated and of approximately the same rank. This irregularity, which results in the loss of the apical cell, is not confined to the root, but is also characteristic of the stem, as was shown previously. Such evidence suggests that *Osmunda* is losing its growth by means of an apical cell, in favor of a type of growth exemplified by some of the higher plants, and more characteristic of massive stems. The evidence is distinctly in support of the views set forth by BOWER (3).

There has been no satisfactory work to date on the histogenesis

of the roots of the Osmundaceae. BOWER describes the meristem of the root of *Osmunda* as being subject to variation, and states:

> since it is hardly possible to determine with certainty from longitudinal sections what is the actual number and form of the initial cells, a full and exact interpretation of the structures in longitudinal sections cannot be expected.

Most of the sections examined by the writer were in support of this conclusion.

Discussion

Nearly a half century ago BOWER (3) made a comparative study of the meristems of a group of families which are included with the Filicales, namely, Hymenophyllaceae, Polypodiaceae, Cyatheaceae, Schizaeaceae, Osmundaceae, and Marattiaceae. His chief contribution was to demonstrate a parallelism in character of all the meristems in these divisions, and to show that in the first named groups the meristems are simple in construction, consisting of an apical cell which exhibits the definite regularity of segmentation also characteristic of the Equisetales. Passing upward through the series, BOWER found that regularity of segmentation is no longer characteristic of the growing points, and that the identity of the single initial is lost as the sequence of segments becomes less definite. The list of families just given is taken from his paper as representing a natural series which grades from the relatively simple to the more complex families of the Filicales.

BOWER regards the Osmundaceae as bridging the gap between the leptosporangiate and eusporangiate ferns. He bases his evidence on a study of the meristems of adult plants, and on certain structural and developmental characteristics of the sporangia. He found that the true leptosporangiate ferns have meristems characterized by an apical cell, that is, by a single initial, and that the sporangium initial is also a single cell. The Marattiaceae have meristems with many initials, in some instances not unlike the growing tips of some modern angiosperms and gymnosperms. The sporangium arises from a group of cells rather than from a single initial. With respect to these characters the Osmundaceae occupy an intermediate position, with their irregular meristems and sporangium initials.

It was thought that perhaps the evidence concerning the meristems of the Osmundaceae might be better evaluated if a study of

the origin of the meristems were made, inasmuch as evidence of the ancestral condition may be found sometimes in the embryonic state. It has been stated already that the stem of the sporeling grows by means of an apical cell which segments in a more or less regular manner. This suggests the leptosporangiate type. As the rhizome ages, irregularities appear and a multicellular meristem frequently results, which resembles the stem tip of certain of the Marattiaceae, more definitely *Marattia* itself. A similar situation has been reported for *M. alata* by CHARLES (**8**), who describes the stems of young sporelings of this species as having a single irregular initial with an early transition to the complex meristem. The root initial prevailingly gives rise to a five-sided apical cell with five cutting faces, but occasionally a tetrahedral apical cell with four cutting faces is developed. This condition is the one found among leptosporangiate forms. As the root develops, the identity of the apical cell is lost and a type of growth appears which suggests the condition found in the Marattiaceae. Thus the evidence derived corroborates the conclusions of BOWER, and in addition suggests that the Osmundaceae are a transitional group; that they are at the present time losing leptosporangiate characteristics and assuming a type exemplified by the eusporangiate fern.

Although the evidence indicates that *Osmunda* is changing from one type of meristem to another, that is, from the leptosporangiate to the eusporangiate type, it throws no light upon the relative antiquity of the two groups. At first thought such evidence might appear to favor the earlier conception of BOWER, that the eusporangiates are the more recent of the groups, but it seems as logical to assume that the Osmundaceae may be passing from a relatively recent leptosporangiate type to the goal attained by modern angiosperms, rather than forward to the eusporangiate level.

The sequence of families listed by BOWER (**3**) was arranged on a basis of comparison of living representatives, and paleobotanical evidence was neglected, as he suggests in some of his later works (**5**). As early as 1891, CAMPBELL (**6**) argued in favor of the eusporangiate ferns as being the more primitive of the two groups. Since that time numerous papers have appeared, many of which have taken paleontological evidence into consideration. To review this literature would be beyond the scope of this paper, and it will suffice to say

that the evidence is now almost preponderantly in favor of the relative primitiveness of the eusporangiate forms. That the Osmundaceae are not contradictory in this respect has been indicated here.

If we may interpret embryonic structures as representing a condition of the adult extant in the past, it seems obvious that *Osmunda* is changing, in that it is apparently becoming more complex. From the description of CAMPBELL (7), *O. claytoniana* seems the least changed of the three species with respect to meristems, inasmuch as it retains the leptosporangiate characters to a greater degree, especially in the root. In this light, excluding anatomical evidence for the moment, it appears feasible to place *O. claytoniana* as the most primitive of the three species, instead of as the most advanced, as suggested by JEFFREY (12). *O. regalis* and *O. cinnamomea* appear to be at about the same phylogenetic level from the standpoint of meristems.

Thus two interpretations may be made on the evidence presented by *Osmunda*. If the Filicales are considered to the exclusion of the spermatophytes, *Osmunda* shows evidence of retrogression and of attaining the primitive eusporangiate type. If the spermatophytes are taken into consideration, *Osmunda* may be thought of as progressing and of approaching the goal exemplified by a number of the angiosperms, a view which is at present more acceptable.

Summary

1. The rhizome of *Osmunda cinnamomea*, when in the sporeling stage, grows by means of a tetrahedral apical cell. As the plant reaches maturity the segmentation of the apical cell becomes progressively less regular, in the majority of cases, until a condition is reached where the identity of the original apical cell is lost, and growth takes place by the activitity of two or more initials.

2. Cells which are destined to become desmogen tissue may be recognized in the second segment from the apical cell.

3. Due to the slow growth of the rhizome and the frequency of leaf gaps, it is extremely difficult if not impossible to refer any portion of the mature rhizome to any definite portion of the segments from the apical cell or group of initials.

4. The pericycle and endodermis apparently have independent origins, although decisive evidence was lacking. The roots arise in

tissue which later gives rise to the pericycle. The undifferentiated endodermis acts as a sort of protective layer to the young root.

5. The number of cells of most young roots is increased for a time by means of an apical cell with five cutting faces. As in the case of the stem, progressive irregularity in segmentation of this cell often results in the formation of two or more initial cells which replace the apical cell.

6. The evidence obtained is in support of the conclusions of BOWER (**3, 5**) that the Osmundaceae occupy an intermediate position between the leptosporangiate and the eusporangiate ferns, but it throws no light upon the relative antiquity of the two groups.

It is a pleasure to acknowledge the aid of Professor W. J. G. LAND, who suggested the problem, helped with the collection of material, and made suggestions concerning the interpretation of the preparations. I am deeply grateful to Professor E. J. KRAUS for his kind criticism of the manuscript and subject matter.

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CORDAITEAN WOOD FROM THE PENNSYLVANIAN OF MICHIGAN AND OHIO[1]

CHESTER A. ARNOLD

(WITH TEN FIGURES)

Among the petrified wood specimens in the Museum of Paleontology of the University of Michigan, two unstudied samples from Michigan and Ohio were recently found. The Michigan specimen was collected by a museum party from a quarry along the south bank of the Grand River, about one mile west of Grand Ledge, in 1925.[2] In the same quarry is a vein of coal a foot or more thick, and a few feet above is a massive sandstone layer bearing Calamites and Artisia casts. Immediately below the coal is a layer of *Stigmaria* shale, and below this and also between the coal and the upper sandstone are shales bearing numerous impressions of foliage, stems, and seeds of common Paleozoic plants. The source of the Ohio specimen is unknown, except that the label reads "Coshocton, Ohio." The specimen appears to have been in the collection for many years, possibly having been obtained by CARL ROMINGER.

But few American Paleozoic woods are known as compared with woods of similar age from other parts of the world. Among those described within recent years, probably the most outstanding are *Calamopitys americana* and *Archeopitys eastmanii*, from the base of the Waverley Shale of Boyle County, Kentucky (13). HOSKINS (5) and REED (11) have recently reported abundant petrified plant material in the Pennsylvanian coal balls of Illinois, which, when more fully investigated, are likely to make notable additions.

Probably the most widespread and abundant American Paleozoic wood is *Callixylon*. *C. newberryi* has long been known from the black shales of Ohio. In 1914, ELKINS and WIELAND (4) described *C. oweni* from the black shales of Indiana, and in 1922 HYLANDER (6)

[1] Paper from the Museum of Paleontology and the Department of Botany of the University of Michigan.

[2] This specimen was communicated to the writer by Professor G. M. EHLERS of the Museum of Paleontology.

described another species from New York. More recent investigations (1) have shown that wood belonging to that genus occurs in abundance just above the Genesee Shale in New York, where four recently described species, *C. erianum*, *C. bristolense*, *C. zalesskyi*, and *C. mentethense*, occur (1); and also in the New Albany Shale of southern Indiana (2). *Callixylon* wood is also known from Kentucky and Oklahoma. In Europe this genus has been reported by ZALESSKY (15) from Russia, and by KRÄUSEL and WEYLAND (8) from Germany.

During the latter half of the nineteenth century, DAWSON (3) described several forms from various Paleozoic horizons of eastern North America, under the generic designations of *Ormoxylon*, *Prototaxites*, *Nematoxylon*, *Aporoxylon*, *Syringodendron*, and *Dadoxylon*. *Prototaxites* (*Nematophyton* of PENHALLOW, and *Nematophycus* of CARRUTHERS), which was thought by DAWSON to be a conifer, is now considered a giant alga.

In 1900, PENHALLOW (10), in a revision of the known American species of *Dadoxylon*, attempted to bring the then existing knowledge of these forms up to date. He described seventeen American Paleozoic species from widely separated localities. One of these he designates as *Dadoxylon*, one as *Pityoxylon* (which according to THOMPSON and ALLIN (14) was misinterpreted), and the remaining fifteen as *Cordaites*. Several of the latter had previously been described by DAWSON as *Dadoxyla*. After the publication of DAWSON's work and before PENHALLOW's revision, KNOWLTON (7) referred many of DAWSON's species to *Cordaites*, since it was not known at the time that primary wood characters are essential for generic identification. Although the total number of species described by DAWSON, KNOWLTON, and PENHALLOW is large, their descriptions and illustrations are inadequate. In many instances the accounts are vague, inaccurate, and unsatisfactory for identification of newly discovered material.

Cordaites michiganensis sp. nov.

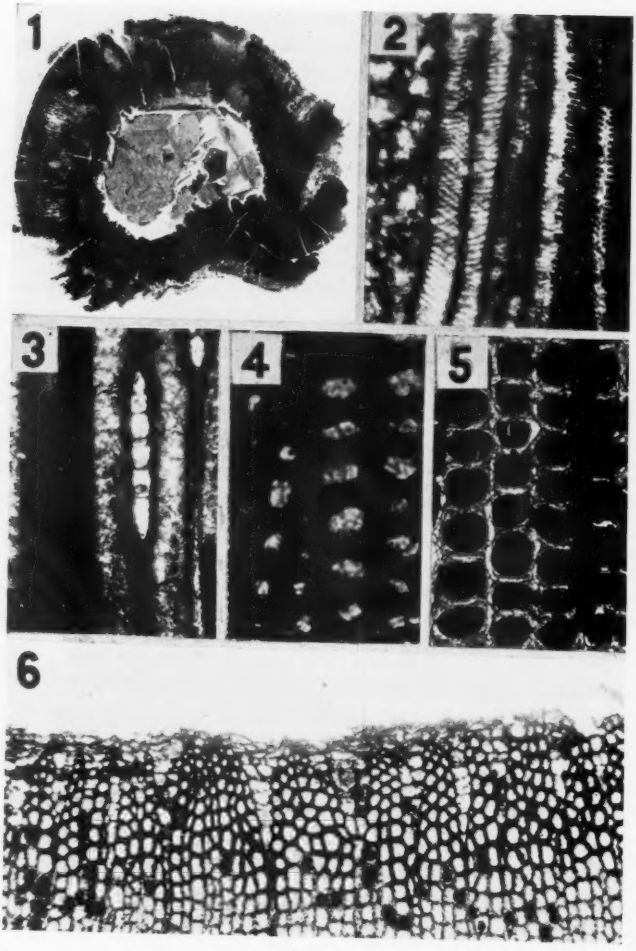
The Michigan specimen is a woody stem about 16 cm. long and 4 cm. in diameter. The large pith region is about 2 cm. across, and surrounding it is a woody zone having approximately the same thick-

ness (fig. 1). Nothing remains of the bark tissues except a layer of disintegrated tissue about 3 mm. thick, which is rather sharply delimited from the adjacent wood. Two nodes about 10 cm. apart are included in the specimen.

MINERALIZATION.—Some interesting features are shown by the mineralization of the specimen. The pith, from which all structure except a few mere traces had disappeared, was replaced by a core of sphalerite. While the prevailing color of the sphalerite is light green, certain portions are purple, suggesting the presence of manganese. These purple portions do not represent separate crystallization, because the cleavage planes, which show clearly under the microscope, pass uninterrupted from one area into the other. The wood region is filled with a mixture of iron pyrites and lime carbonate, the former being present in greater quantity (fig. 1). In some places both the cell walls and the lumina contain the pyrites. In other places the pyrites has infiltrated the walls but the lumina remain clear (fig. 4). In other places the reverse condition is shown, the lumina being filled with pyrites with the cell walls calcified and consequently clear (fig. 5). Then in certain small areas the petrifying mineral is entirely lime carbonate, no pyrites being present. It is only in these portions that the specimen can be studied satisfactorily by transmitted light.

In some portions of the stem the cells are badly decayed. The disappearance of structure in the pith is especially unfortunate, since it not only renders a determination of its structure impossible, but the decay and replacement have obscured some of the features of the structure of the primary wood. In transverse section the secondary wood could be studied satisfactorily in the unpyritized portions. The preparation of satisfactory radial and tangential sections was difficult, owing to the combined problem of orientation of the specimen for cutting and the chance of the cut passing through a region consisting largely or entirely of poorly preserved tissue.

ANATOMY.—The stem is gymnospermous and without growth rings. The secondary wood tracheids average 35 μ in radial diameter, are regular in size and shape, and in general are square or slightly rectangular (figs. 5, 6). The square aspect may be increased by partial decay of the secondary walls.



FIGS. 1–6.—*Cordaites michiganensis* Arnold sp. nov.: Fig. 1, cross-section of stem, showing dark pyritized portions and calcified areas which are lighter; ×1-3/8. Fig. 2, radial sections through primary wood showing closely wound spiral structure; lumen of tracheid at right is pyritized; ×180. Fig. 3, tangential section showing typical ray; ×180. Fig. 4, transverse section showing pyritized cell walls and calcified lumina; ×180. Fig. 5, transverse section showing pyritized lumina and calcified walls; ×180. Fig. 6, transverse section of portion of primary wood showing broadening of rays and consequent narrowing of primary wood segments as they approach pith; ×45.

So far as can be determined, the pits are confined to the radial walls of the tracheids. Decay renders them obscure, but in places of favorable preservation they are seen to be hexagonal and alternately arranged. They occur mostly in two rows per tracheid, but sometimes there are three or four.

The rays, which are small and narrow, vary in height from two to ten cells (fig. 3). For the most part they are uniseriate, but occasional partial biseriation near the middle of the ray is caused by two cells lying side by side. The biseriation seldom or never occurs for their entire height. The ray cells may be slightly higher than wide but the difference is slight, and they are variable in length, ranging from the cross diameter of one to that of four tracheids. The cross diameter of a ray cell is slightly less than that of a tracheid.

As the rays approach the pith they gradually broaden, becoming narrowly fan-shaped. Consequently the xylem masses between these rays become narrowed to a point at the margin of the pith (fig. 6). These narrow wedges of the inner wood represent the primary xylem, which in this case is not sharply defined from the secondary wood to which it is joined. At the tips of these wedges the xylem elements are small, but they become progressively larger farther back. At the broadest place the wedges vary in width from one to seven or eight cells. These wedge-shaped masses consist of tracheids of the closely wound spiral type (fig. 2), which extend radially for a distance of eight to ten tracheids, as near as preservation of the material permits one to judge, and in some places a brief zone of one or two cells shows reticulated structure just before pitting commences.

Because of pyritization and decay at several critical places, little can be determined concerning the origin and departure of the traces, although they appear in some of the more favorable instances to consist of a double strand. Consequently it is impossible to determine with any degree of certainty whether they possess any centripetal primary wood. For this reason it is unadvisable to refer this form to *Mesoxylon*, which, according to MASLEN (9), is to be distinguished from *Cordaites* only by the presence of centripetal wood on the downward extension of the leaf trace at the margin of the pith.

In default, therefore, of this one character which is essential in

identifying a form as *Mesoxylon*, and because of the close resemblance of this specimen to forms originally described as *Cordaites*, it is considered advisable to refer it to that genus. According to RENAULT (12), the primary xylem region of *Cordaites* consists of a series of inward projecting wedges separated one from the other by the broadening of the rays as they approach the pith. This feature was also observed in *Mesoxylon multirame*, when comparing my specimen with a specimen of that species. MASLEN states that possibly the majority of the stems previously described as *Cordaites*, have the *Mesoxylon* structure, suggesting the possibility of RENAULT'S *Cordaites* being a *Mesoxylon*. The centripetal wood might easily have been overlooked, since at the time he wrote the primary wood was not yet regarded as important for generic identification. At any rate, RENAULT'S *Cordaites*, *Mesoxylon*, and my form show marked similarity in primary wood structure, and the apparent differences might be due to preservation. RENAULT'S description of the wood of *Cordaites* is brief and lacks many desirable details. Also it should be noted that the distinguishing feature of *Mesoxylon* is merely the presence of a structure not mentioned by RENAULT and which he probably overlooked. On the other hand, it is true that during Paleozoic times the cordaitalean complex was a large and diversified group of plants, and the occurrence of two forms differing only with regard to the feature under consideration is easily within the range of possibility.

The large pith (whether or not it was discoid could not be determined), the alternate-multiseriate pitting, and the narrow rays are features also indicative of *Cordaites*.

The Michigan wood does not seem to agree closely with any of the American cordaitean forms described by DAWSON (3) or PENHALLOW (10). It appears to differ in many respects from *Cordaites brandlingii*, the common European form, and it is unlikely to have affinities with the numerous forms from the upper Coal Measures or Permian formations. Since this specimen is older than most of the other known forms (it being from the lower Coal Measures), and because of its isolation (the nearest previously described forms are from Illinois and Ohio, and they differ from it), it appears advisable to describe it as a new species.

Cordaites michiganensis sp. nov.—Tracheids averaging 35 μ in diameter, square to slightly oblong, regular in size and shape. Bordered pits alternately arranged, hexagonal, chiefly in 2 rows per tracheid. Rays narrow, low, and scattered; uniseriate or partly biseriate in the middle. Ray cells about equal in height and width, narrower than the tracheids, in length equal to the cross diameter of 1–4 tracheids. Rays broader as they approach the pith, dividing the primary wood region into wedge-shaped segments.

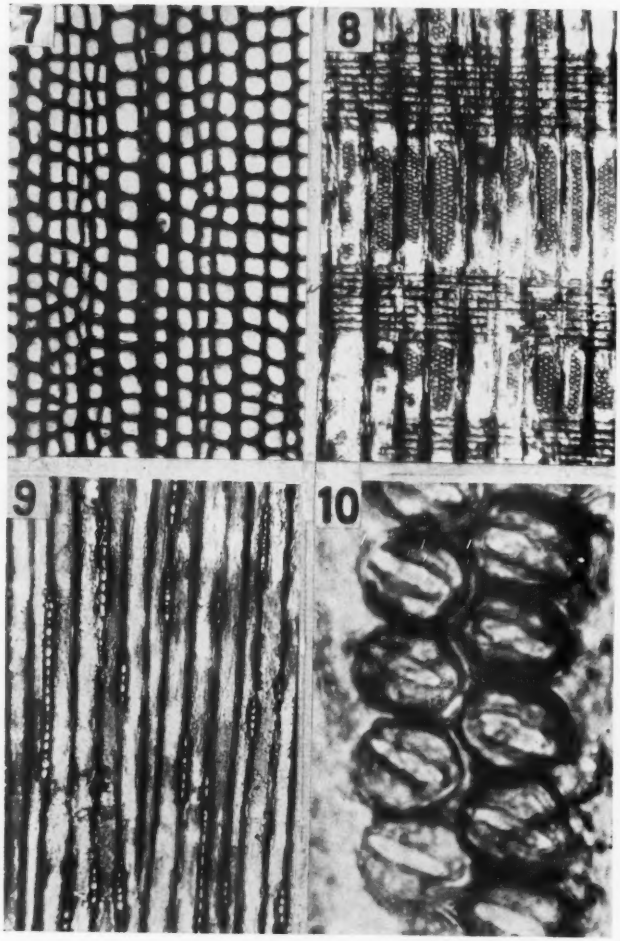
Horizon, probably Saginaw, lower Pennsylvanian. Locality, Grand Ledge, Michigan. Type no. 12405, Museum of Paleontology, University of Michigan.

Dadoxylon romingerianum sp. nov.

The Ohio specimen consists of a piece of well preserved silicified secondary wood measuring about 2.5×5×8 cm. Judging from the convergence of the radial faces of the block it apparently came from a large trunk, at least 6 inches from the center. Since there is no information concerning the source of this specimen, other than the label which reads "Coshocton, Ohio," its Pennsylvanian age is only inferable.

The wood, which is gymnospermous (fig. 7), shows no growth zones in the 5 cm. of its radial extent. The tracheids are large, averaging 60–70 μ in radial diameter, but varying from 45 to 90. The radial diameters are somewhat greater than the tangential diameters, which are variable. Some of the tracheids are nearly round, with all diameters approximately equal.

The bordered pits are alternately arranged in 2–5 rows per tracheid. Two or three rows are most commonly observed (fig. 8). Tracheids bearing five rows of pits are rather rare. The pits are of the hexagonal type but are slightly compressed vertically, thus rendering the vertical diameter somewhat less than the transverse one, which varies from 12 to 18 μ (fig. 10). The pit orifice is the diagonal slit commonly observed in woods of this type, but because of the narrowed vertical diameter of the pit the orifice is inclined at an angle somewhat less than 45° from the horizontal. In some of the more closely crowded pits the orifice lies nearly at right angles to the vertical axis of the tracheid.



FIGS. 7–10.—*Dadoxylon romingerianum* Arnold sp. nov.: Fig. 7, transverse section; ×55. Fig. 8, radial section showing characters of rays and pitting; ×65. Fig. 9, tangential section showing narrow rays in contrast to broad tracheids; ×65. Fig. 10, bordered pits in radial section, highly magnified, showing slight vertical flattening of bordered pits and low inclination of pit orifice; ×1300.

The rays, most of which are less than twenty cells high, may vary from two to forty cells (fig. 9). They are mostly uniseriate but partial biseriation occurs frequently. The biseriation may extend vertically for about six cells in some instances, but ordinarily it consists merely of two cells lying side by side near the middle of the ray. The ray cells are thin-walled and some of them have dark contents. The side walls are pitted, there being usually one or two pits between the ray cell and each adjacent tracheid. The ray cells range from 15 to 18 μ in width, with their vertical and horizontal diameters about equal. In length they are approximately equal to the cross diameters of 4–6 tracheids. The ray cells of small diameter in connection with the large tracheids provide a characteristic which is obvious and appears to distinguish this form (fig. 8).

In the absence of the primary wood the exact generic affinities of this specimen are indeterminable, and must remain an open question, so it is considered well to adhere to the established custom of referring such problematic material to *Dadoxylon*. While showing the same general *Dadoxylon* (or cordaitean) type of structure as the Michigan specimen just described, the two specimens are specifically different, and aside from very general features there are no indications of their generic identity. Several forms, of which *Mesoxylon*, *Callixylon*, and *Poroxylon* are examples, are cordaitean and would probably be considered congeneric were it not for the distinctive characters of the primary wood.

The characters which serve to distinguish the specimens may be contrasted thus:

CORDAITES MICHIGANENSIS	DADOXYLON ROMINGERIANUM
Tracheids regular, average diameter 35 μ.	Tracheids irregular, average diameter 60–70 μ.
Bordered pits mostly in 2 rows, height and breadth equal.	Bordered pits mostly in 2 or 3 rows compressed vertically.
Rays low, seldom more than 10 cells high.	Rays higher, frequently more than 10 cells high.
Rays only slightly narrower than tracheids.	Rays much narrower than tracheids.

In cell dimensions, biseriation, etc., the rays of the two specimens are similar. As noted, however, they are somewhat higher in the

Ohio specimen, and being associated with rather large tracheids the ray cells appear small in proportion, a feature not so obvious in the Michigan form.

The Ohio specimen, in comparison with forms previously described from Ohio, shows little evidence of near affinity. Perhaps the closest approach is *Cordaites matariarum* Daws., which, according to PENHALLOW (10), has tracheids $45 \times 70\ \mu$ and narrow rays. The pits of this form, however, do not show the vertical shortening which is such a conspicuous feature in our specimen. *Cordaites ohioense* Daws. (10) and *Callixylon newberryi* (Daws.) Elkins & Wieland (4) show no basis for comparison. In describing this specimen as a new species it is certainly appropriate to name it in commemoration of the well known geologist of the last century, CARL ROMINGER, in whose wood collection this specimen was found.

Dadoxylon romingerianum sp. nov.—Tracheids averaging 60–70 μ in diameter, irregular in size and shape. Bordered pits alternately arranged in 2–5 (mostly 2–3) rows per tracheid, compressed vertically, horizontal diameter 12–18 μ; orifice a diagonal slit. Rays narrow, few to 40 cells high, uniseriate or partly biseriate in the middle. Rays long, height and width about equal, much narrower than adjacent tracheids. Pits on side walls of ray cells 1 or 2 per tracheid.

Age, probably Pennsylvanian. Locality, Coshocton, Ohio. Type no. 12406, Museum of Paleontology, University of Michigan.

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SPECIALIZATION IN SECONDARY XYLEM OF DICOTYLEDONS

III. SPECIALIZATION OF LATERAL WALL OF VESSEL SEGMENT

FREDERICK H. FROST[1]

(WITH EIGHT FIGURES)

Introduction

In 1894 BOODLE and WORSDELL (4) advanced the hypothesis that scalariform lateral pitting in the vessels of dicotyledons originates by fusion of horizontal rows of circular pits. In recent years JEFFREY (9), BLISS (3), and MACDUFFIE (10) have elaborated a similar concept. On the contrary, THOMPSON (11), BROWN (5), BAILEY and TUPPER (2), and BAILEY (1) have contended that scalariform lateral pitting is primitive and gives rise by specialization to the circular type. In view of the fact that both groups of investigators agree that the transitional types of pitting between these two extremes are phylogenetically significant, the main point at issue is the problem of determining the direction of the specialization. This paper attempts to settle this question, based on a study of a wide range of dicotyledons and by evidence independent of the transition itself. The nature of the pitting between vessels and parenchyma is also discussed.

LATERAL INTERVASCULAR PITTING

The type of pitting between adjacent vessel segments is divisible into four classes: scalariform (fig. 1), transitional (fig. 2), opposite (fig. 3), or alternate (fig. 4). Scalariform lateral pitting is characterized by the oblong-elongate form of the pits in surface view. Transitional lateral pitting, as the name implies, is a combination of scalariform and opposite pitting. Scalariform and transitional lateral pitting occur in many families of dicotyledons. The scalariform type is not a "phenomenon of rare occurrence" BLISS (3). Opposite pitting is distinguished from alternate by the orientation of the pits.

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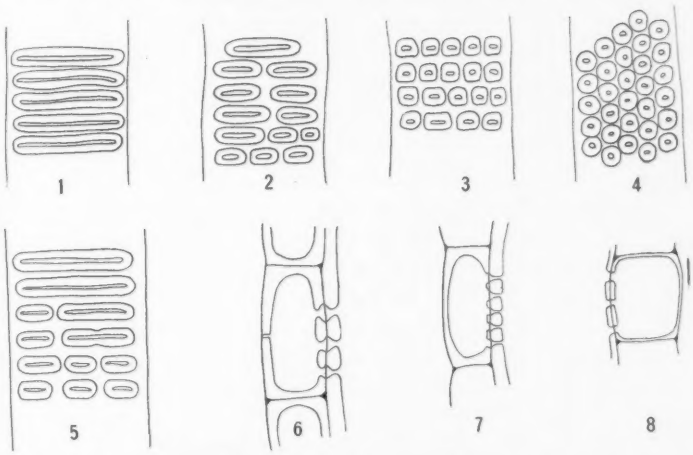
In the former the pits are arranged in horizontal rows, whereas in the latter they form diagonal (but not horizontal) rows of contiguous pits. Opposite pits tend to be oval or rectangular, and alternate ones round or hexagonal; both types are classified under the heading of "circular" pits. Circular pitting characterizes the vessel segments of about 80 per cent of the existing species of dicotyledons.



FIGS. 1–8.—Fig. 1, scalariform lateral pitting from *Hedycarya arborea;* fig. 2, transitional lateral pitting from *Talauma ovalis;* fig. 3, opposite lateral pitting from *Liriodendron tulipifera;* fig. 4, alternate lateral pitting from *Planera aquatica;* fig. 5, transitional stages in formation of circular pitting from *Magnolia kobus;* fig. 6, fully bordered vessel-ray pits from *Sassafras variifolium;* fig. 7, half-bordered vessel-ray pits from *Hicoria ovalis;* fig. 8, non-bordered vessel-ray pits from *Bombax malabaricum.*

It was shown in the first (**7**) and second (**8**) papers of this series that specialization of the vessel results in a gradual reduction in length of the vessel segments. By the application of the method of correlation, if circular pitting is more primitive than scalariform, it follows that the vessel segments which are characterized by circular pitting will be of greater average length than the scalariform-pitted segments. On the contrary, if the scalariform type of pitting is primitive the reverse will be the case. Table I shows the actual correlation.

The numerical difference between the means of the scalariform

and transitional groups is not statistically significant, due to the small number of cases and the relatively slight distinctions between the two modes of pitting. The differences between the means of the scalariform and transitional groups and the opposite group, however, is statistically significant since a chance difference of this magnitude would not occur once in ten thousand times. The mean of the alternate group is likewise stable. The obvious conclusion is that scalariform lateral intervascular pitting is primitive, and that the sequence should be read from scalariform, to transitional, to opposite, culminating in the highly specialized alternate arrangement of circular pits.

It has been shown in table IV of the first paper (7), and by BAILEY and TUPPER (2), that the stages of the transition from scalariform to

TABLE I

TYPE OF LATERAL PITTING	NO. OF SPECIES	LENGTH OF VESSEL SEGMENTS (MM.)
I. Scalariform	15	1.13
II. Transitional	28	1.07
III. Opposite	33	0.79
IV. Alternate	183	0.46

simple perforations correlate with the stages of the transition from scalariform to alternate intervascular pitting. This is an important fact, since the followers of the fusion hypothesis hold that scalariform perforation is ancestral to the simple perforation. If the fusion hypothesis is still maintained despite this evidence, therefore, it will be necessary either to abandon the concept that the scalariform perforation is more primitive than the simple perforation, or to admit that the characteristics of a cell type or homogeneous tissue can develop independently of one another. If the existence of phylogenetic correlation is denied, in the face of the many proofs of its reality, the solution of the problem becomes an impossibility. It should be noted that the doctrines of recapitulation and of conservative regions would be invalidated if phylogenetic correlation between tissues were non-existent.

Palingenetic sequences from scalariform to opposite pitting are frequent, and may be found in the variability presented by the

species of a genus, the variability of the vessel segments of a species, in the transitional region between the protoxylem and the secondary xylem of many of the less specialized dicotyledons, and not uncommonly on the lateral walls of an individual vessel segment. Fig. 5 (*Magnolia kobus*) illustrates the breaking down of scalariform pitting as reflected in positional sequence on the lateral wall of the vessel segment. In the majority of dicotyledons this transition has reached the alternate arrangement, since the alternate condition is by far the most common type. This is particularly true of species found in temperate regions.

As the vessel segment is reduced in length during specialization, it frequently increases in width. In general the breaking down of the scalariform intervascular pitting occurs before or in correlation with this increase in diameter of the vessel. Occasionally, however, the scalariform sculpture persists and in such instances the scalariform pits are apparently drawn out horizontally. *Rhizophora mangle*, *Bruguiera gymnorrhiza*, and *Vitis* show this condition. The vessels are large in cross-section and show decided evidences of specialization. The lateral pitting has remained scalariform, however, and the pit orifices are narrow.

Vessel-parenchyma pitting

The type of pitting found between vessels and parenchyma is not, as has been suggested (**9**), controlled by the orientation of the parenchymatous elements. In the majority of cases the pitting found between parenchyma and vessels is similar in type to the intervascular pitting of the species under consideration. That is, the pits may be scalariform, transitional, opposite, or alternate. In the 260 species studied in this connection, it was found that in 85 per cent the vascular-parenchyma pitting was of the same type as the intervascular pitting. As a rule scalariform and transitional vessel-ray pits are oriented with their long axis parallel to the direction of the ray. In some species of *Quercus* and *Castanea*, however, the long axis is vertical. Transitional types between the horizontal and vertical position are not infrequent. The correlation between vessel segment length and the nature of the vessel-ray pits is shown in table II.

The correlation between specialization in the length of the vessel

segment and the evolutionary development of the vessel-ray pitting is even more striking in this case than the correlation shown in table I. The average length for each type of pitting, however, is slightly lower for each group in table II. This difference is probably significant, since the intervascular pits specialized more rapidly than the vascular-parenchyma pits, as indicated by the fact that in 85 per cent of the species, which show a difference in pitting in these two situations, the intervascular pits are more highly specialized than the vascular-parenchyma pits. This slightly slower rate of development would tend to reduce the means in each case.

The situation in *Trochodendron* and *Tetracentron* is interesting in this connection. In both of these vessel-less genera the first formed tracheids of the annual ring have the primitive scalariform lateral

TABLE II

VESSEL-RAY PITTING	NO. OF SPECIES	LENGTH OF VESSEL SEGMENTS (MM.)
I. Scalariform	13	1.11
II. Transitional	42	0.96
III. Opposite	49	0.74
IV. Alternate	156	0.43

pitting. Where tracheids of this type adjoin ray parenchyma the pitting is transitional to opposite; scalariform pits are found near the pith but are of rare occurrence in the later growth rings. The type of pitting found between the circular pitted tracheids and the ray parenchyma is opposite or alternate, as one would expect. It is apparent that the specialization of the tracheid-ray pits proceeded more rapidly than the specialization of the tracheid pitting, the reverse of the usual procedure when the ray is in contact with a vessel segment.

The writer (**6**) has shown that vessel-parenchyma pits may be fully bordered, half-bordered, or non-bordered (figs. 6–8).

Correlating these types with an established evolutionary sequence in order to determine the course of development presents several difficulties. In well prepared radial and tangential sections it is impossible in many cases to find more than three or four pits which can be classified definitely, and in such instances the dominant type can-

not be determined. In other cases the vessel-ray pits are so minute that it is unsafe to reach a decision as to their nature. While these difficulties have made it impossible to work out the details of the evolutionary development of the bordered pit, it has been possible to determine certain facts regarding the specialization. Table III shows the correlation between the nature of the border and the length of the vessel segment.

TABLE III

Type of border	No. of species	Vessel segment length (mm.)
I. Bordered	58	0.81
II. Half-bordered	170	0.56
III. Non-bordered	23	0.53

The correlation between the type of border and the type of vessel-parenchyma pit is given in table IV. If this evidence is assumed to be statistically reliable, the direct interpretation is that the fully bordered pit is primitive and that the sequence should be read from

TABLE IV

Type of pitting	No. of species	Percentage of species with fully bordered pits
I. Scalariform and transitional	54	46
II. Opposite	49	35
III. Alternate	147	11

fully bordered, to half-bordered, to simple or non-bordered. Since it is relatively easy to distinguish the species possessing half-bordered and fully bordered vessel-parenchyma pits from those with simple pits, it is clear that the non-bordered condition is a specialization of either the bordered or half-bordered pit. This is indicated by the fact that the average length of the vessel segments of species with fully bordered and half-bordered vessel-parenchyma pits is higher than the average for species with simple pits, since the non-bordered type is not found in the truly primitive woods, and since primitive plants in general are characterized by the half-bordered or bordered pit in this situation.

The relative position of the bordered and half-bordered types is not so clear, since the separation into half-bordered and bordered groups cannot accurately be accomplished. Although tables III and IV indicate that the fully bordered pit is the most primitive, there are undoubtedly fully bordered species listed as half-bordered and half-bordered species listed as fully bordered. In addition to this complication, the specialization of the bordered pit is not highly correlated with the development of other characteristics of the vessel segment. This results in woods with bordered and half-bordered vessel-parenchyma pits which are highly specialized in other respects.

The most probable interpretation of these data is that the pro-angiospermous stock possessed bordered pits in this situation and that the fully bordered type persisted after the introduction of the vessel. Soon after, the half-bordered type originated by the loss of the border on the wall of the living cell, and later the loss of the remaining border produced the non-bordered vessel-parenchyma pit. A delay in the introduction of the half-bordered pit, possibly due to environmental factors, would then account for the highly specialized woods with fully bordered pits.

Another alternative is that the half-bordered pit is primitive and was characteristic of the pro-angiospermous stock. If this were the case the bordered pit must have originated practically simultaneously with the vessel, since the fully bordered type is characteristic of many of the more primitive existing dicotyledons. Subsequent specialization of the bordered pit might then produce the half-bordered and non-bordered types, or the simple pit might originate directly from the primitive half-bordered pit. While this explanation is possible it does not appear very probable.

Unfortunately the preservation of the older fossils with scalariform tracheids is not such as to allow a distinction to be made between the bordered and half-bordered vessel-parenchyma pitting, and at the present time the true nature of these pits is doubtful. The problem will therefore remain unsettled until satisfactory palaeontological material establishes which of these alternatives is the correct interpretation.

The question of tertiary spiral thickenings is of interest. Of the

260 species studied, 41 had spiral thickenings in the vessels of the secondary wood. The average length of the vessel segments of these 41 species is 0.46 mm., which is less than the average length of vessel segments in general, and shows that introduction of the spiral is an evidence of decided specialization. Spirals normally occur in woods with porous perforations, but may be found occasionally in scalariform and scalariform-porous woods (*Magnolia*). In the latter case the vessels always show several markedly specialized features. The vessel segments are short, the lateral pitting opposite, the apertures of the perforation non-bordered and large, the number of bars reduced in number, or the walls considerably thickened. The writer has not observed spirals in such primitive woods as *Schima wallichii* or *Dillenia philippinensis*.

Summary

1. Scalariform intervascular pitting is primitive in the organization of the vessel segments of the dicotyledons.

2. Specialization of the scalariform pit produces transitional and opposite pitting. The re-arrangement of opposite pits gives rise to the highly specialized alternate arrangement of intervascular pits.

3. Vessel-parenchyma pitting may be scalariform, transitional, opposite, or alternate. The evolutionary development is the same as in the development of intervascular pitting.

4. Vessel-parenchyma pits may be fully bordered, half-bordered, or non-bordered. The fully bordered pit appears to be primitive and to give rise during specialization to the half-bordered and the non-bordered type.

5. The introduction of tertiary spirals in secondary vessel segments is an evidence of specialization. Spirals do not occur in the most primitive dicotyledonous woods.

In conclusion, the writer expresses his appreciation to Professor I. W. BAILEY for many suggestions and the use of his extensive collections.

CUMBERLAND MILLS
MAINE

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DEVELOPMENT OF FLORAL ORGANS OF THE SOY BEAN[1]

ARTHUR T. GUARD

(WITH SIXTEEN FIGURES)

Development of the floral organs of *Soja max* Piper has been studied, from the appearance of the knoblike protuberance which forms the receptacle to the mature flower, in order to determine the sequence of appearance of the organs and the course of their development to maturity. Only the Manchu variety was used. The plants which furnished material for this investigation were grown in the garden, since attempts to grow material in the greenhouse yielded only poorly developed buds and flowers which never fully opened.

Rawlin's formula of formalin-acetic-alcohol, Schaffner's chromo-acetic acid, and Flemming's medium solution were employed as killing and fixing agents. Either Delafield's haematoxylin counterstained with safranin or Flemming's triple was used as the stain.

GROSS MORPHOLOGY

The flowers of the soy bean are borne in axillary (rarely terminal) racemes (fig. 1), usually of five to sixteen flowers each. PIPER and MORSE (**4**) report instances of as many as thirty-five flowers in a single inflorescence. The perfect, polypetalous, zygomorphic flower is approximately 6 mm. in diameter when fully opened.

The calyx is tubular, terminating in five unequal lobes of which the largest is anterior, the next two lateral, and the two smallest obliquely posterior. It is persistent, but grows little during the development of the fruit. The corolla consists of five distinct petals (fig. 4). The largest is posterior, the two next in size (wings) are lateral, and the two keel petals are obliquely anterior. Although the margins of the two keel petals touch, there is no coalescence, as is the case in the keel petals of the flowers of many legumes.

The androecium consists of ten diadelphous stamens (fig. 2), all

[1] Contribution from the Biology Department of Purdue University, Lafayette, Indiana.

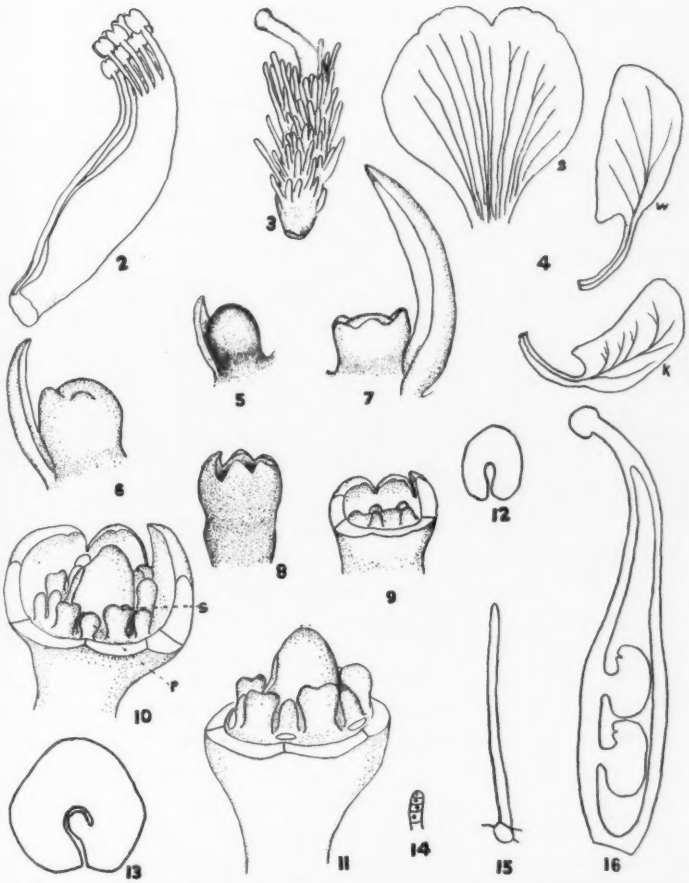
of which are separate at first, but later the filaments of nine of them are elevated as a single structure by the pushing up of a basal region of meristematic tissue, leaving the posterior stamen separate. The single pistil (fig. 3) is already covered with trichomes by the time the flower opens. These are of two kinds, one (fig. 15) a unicellular solitary structure, the other (fig. 14) a multicellular uniseriate trichome usually of four cells, occasionally of five or six. The style, which is about half the length of the ovary, curves backward toward

FIG. 1.—Inflorescence of soy bean, a raceme (usually axillary, occasionally terminal)

the posterior stamen, and is surmounted by a knoblike stigma which is receptive for pollen at the time the flower opens.

EMERGENCE OF ORGANS

The first sign of a flower in the soy bean is the appearance of a knoblike primordium in the axil of a bract (fig. 5) before this has reached its mature size. The anterior calyx lobe arises on the anterior side of this primordium (fig. 6), followed by the two lateral lobes, and later by the two obliquely posterior lobes (fig. 7). Early in their development the bases of the calyx lobes broaden until they meet, and finally the whole basal region is pushed up as the calyx tube (fig. 8). The primordia of the petals (fig. 9) are alternate with the lobes of the calyx, and more distal on the floral axis. The two obliquely anterior or keel petals appear first, next the two lateral ones (wings), and finally the posterior petal. This sequence of emergence



FIGS. 2–16.—Fig. 2, Androecium showing nine united and one free filament; ×10. Fig. 3, pistil with ovary densely covered with trichomes as it appears as flower opens; ×10. Fig. 4, part of corolla; ×10. Fig. 5, young flower primordium appearing in axil of bract; ×90. Fig. 6, flower primordium with calyx lobes beginning to emerge; ×90. Fig. 7, flower primordium with only primordia of calyx lobes present (no other parts yet appeared); ×90. Fig. 8, entire calyx region pushing up to form calyx tube; ×90. Fig. 9, flower primordium with part of calyx cut away to show five petal primordia just emerging; ×150. Fig. 10, more advanced stage than fig. 9, with part of calyx cut away to show primordia of petals (*p*), stamens (*s*), and pistil; ×200. Fig. 11, flower bud with calyx and petals cut away to show position and relative size of two cycles of stamens and

corresponds with that observed by PAYER (3) in *Trifolium ochroleucum*. GOEBEL (2) reports this to be the usual order of emergence of floral organs in the Leguminosae.

While the petal primordia are still very small, the primordia of the outer cycle of stamens (fig. 10) appear. The five individual members of this cycle are opposite the calyx lobes and alternate with the petals. The sequence of appearance is also from anterior to posterior on the receptacle.

By the time the last primordium of this cycle is visible, a second cycle of stamens, its members alternating with those of the first, begins to develop in the same order. That they are slightly distal to the first cycle is noticeable in the early stages of development (fig. 11), but later the inner cycle appears to merge with the outer, as a result of the development of the tissue which is basal to both cycles. The presence of two cycles of stamens, one slightly preceding the other, was also observed by PAYER (3) in *Trifolium ochroleucum*, WESTGATE and his co-workers (5) in *Trifolium pratense*, and COE and MARTIN (1) in *Melilotus alba* and *M. officinalis*. Almost simultaneously with the emergence of the last cycle of stamens, the single primordium of the carpel appears. This develops rapidly and early becomes the largest of the primordia (fig. 11).

GROWTH OF ORGANS

Before there is any differentiation in either stamens or pistil, the calyx during its development forms a protective covering for the other organs. At this time the trichomes are beginning to form on the calyx, and these continue to increase in number until the calyx is very hairy. These trichomes are similar to those on the pistil and leaves. The growth of the petal primordia is very slow until the microsporangia begin to develop in the anthers, when the petals begin to grow rapidly, pushing the calyx lobes apart, and the flower opens.

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pistil; ×200. Fig. 12, diagrammatic cross-section of young pistil with margins not yet coalesced; ×90. Fig. 13, diagrammatic cross-section of pistil showing ovule beginning to develop on one margin; ×150. Fig. 14, multicellular trichome from ovary; ×90. Fig. 15, unicellular trichome from ovary; ×90. Fig. 16, diagrammatic vertical section of pistil of mature flower showing position and orientation of ovules.

Early in its development each of the stamens is differentiated into an anther and a filament. The filaments continue to develop separately until the tetrads are beginning to round into microspores. At this time the tissue basal to the two cycles pushes up nine of them as a single structure, leaving the tenth stamen free. This free filament elongates, however, so that its anther remains on a level with the anthers of the other four stamens of the inner cycle (fig. 2). Dehiscence of the anthers occurs at the time the flowers open.

The pistil early in its development is a leaflike carpel (fig. 12), folded along its midrib with its margins scarcely touching. Before these margins coalesce, protuberances (which later develop into ovules, fig. 13) are produced alternately on the inner surface of these carpel margins. In its development the pistil lags somewhat behind the stamens, as WESTGATE and his co-workers report for *Trifolium pratense*, and COE and MARTIN found in *Melilotus alba* and *M. officinalis*.

The ovules are campylotropous (fig. 16) at maturity, with the micropyle directed toward the distal end of the ovary. COE and MARTIN found a different orientation of the ovules in *Melilotus alba* and *M. officinalis*, in which the micropyle is facing the basal end of the ovary. There are usually two or three ovules in each ovary, but occasionally only one or rarely four are found. These ovules develop simultaneously. The style grows slowly until the flower is ready to open, when it elongates rapidly until it is about two-thirds the length of the ovary. The stigma is a knob covered with papillae.

Summary

1. The mature flower of the soy bean consists of a calyx tube with five unequal lobes, five distinct petals, ten stamens (nine united and one free), and a single carpel. Both unicellular and multicellular trichomes are found on the calyx and pistil.

2. The flower primordium appears first as a protuberance in the axil of a bract. The order of emergence of the floral organs is sepals, petals, outer cycle of stamens, inner cycle of stamens, pistil. The order of emergence within each cycle is from the anterior to the posterior side of the receptacle.

3. Development of the petals is slow at first, but later becomes

rapid. The stamens are free at first; later nine become united leaving one of the inner cycle free.

4. The one to four campylotropous ovules develop simultaneously, and the micropylar end is directed toward the distal end of the ovary.

The writer wishes to express grateful appreciation to Professor E. J. KOHL and to Dr. M. W. GARDNER for their suggestions and criticisms during this investigation and preparation of the manuscript.

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BRIEFER ARTICLES

A NEW SPECIES OF GRAMA GRASS

(WITH FOUR FIGURES)

Among a number of grasses which were sent to the writer by Mr. BURT ENGLISH of Fort Sill, Oklahoma, for identification was a strange specimen of *Bouteloua*. A search of literature on the genus failed to reveal a description of it. In many respects it resembles *Bouteloua hirsuta* Lag., and in the National Herbarium it has been classified as that, but the descriptions of the two grasses are not the same. The key in GRIFFITHS'[1] work on grama grasses has no place for it. His description of *B. hirsuta* does not fit, and drawings of the same species (p. 374, fig. 34) do not conform to it.

VASEY perhaps recognized this as a variety of *Bouteloua hirsuta*, but it was never validly published. Specimen no. 883032 of the National Herbarium is labeled *Bouteloua oligostachya* var. *major* Vasey, *E. P. Stiles*, Austin, Texas, 1884. This was misidentified and had been filed under *B. hirsuta*. On the mount there are the drawings of some spikelet parts, among which is a drawing of a sterile pedicel with a tuft of hair on one side at the top. The specimen on this mount is similar to the one sent in by Mr. ENGLISH. The drawing, however, should have shown a tuft of hair on each side at the top of the sterile pedicel. VASEY[2] shows a drawing of *Bouteloua hirsuta* var. *major*, but this drawing is not of the same kind of grass as that collected by *Stiles*.

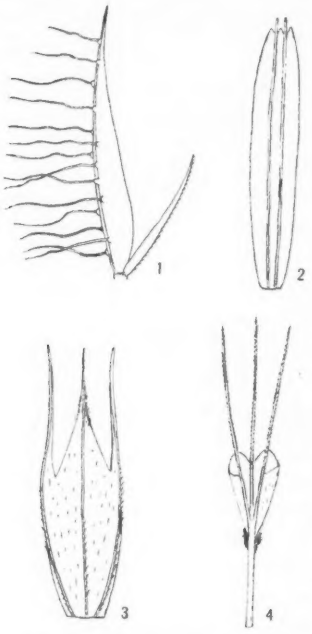
Since none of the drawings describe the plant, and since there is no heretofore published description of it, it seems proper to describe it. The differences between this plant and other members of the genus appear to be sufficient to make it a new species (figs. 1–4).

Bouteloua pectinata sp. nov.—Perennis. Culmi fertiles 4–7 dm. alti, erecti, simplices, glabri; culmi steriles breves numerosique; folia plurime basalia; vaginae glabrae ac laeves, fauce ciliatae, inferiores internodiis longiores, laxae, striatae; ligula anulus ciliatus non perspicuus; laminae usque ad 25 cm. longae, 1–2.5 mm. latae, planae vel apicem versus invo-

[1] GRIFFITHS, D., The grama grasses. Cont. U. S. Nat. Herb. 14:372–375. 1912.

[2] VASEY, GEO., U.S. Dept. Agric. Div. Bot. Bull. 12. 1890.

luto-attenuatae, erectae, flexuosae, rigidulae, margines versus crassatae, glabrae; margines minute scabrae, laminarum inferiorum basim versus papilloso-hispidae; spicae 3 vel 4, 3–5 cm. longae, leviter curvatae, pedunculo pubescenti 4–5 mm. longo gestae; rachis spicula rudimentaria terminans; spiculae 40–60, pectinatae, 5–6 mm. longae; glumae lanceolato-acuminatae, uninerviae, inaequales, puberulae, prima 4 mm. longa nervo scabra, secunda 5–6 mm. longa (arista brevi adjecta) tergo pilis circ. 2 mm. longis perspicue papilloso-hirsuta, papillis longe arista extendentibus; lemma aristis adjectis circ. 6 mm. longa, tergo hirsuta, triaristata; palea circ. 4 mm. longa, binervia, nervis in aristas breves desinentibus; pedicellus sterilis apice crinibus bicristatus.



Figs. 1–4.—*Bouteloua pectinata:* glumes, palea, lemma, and sterile pedicel with rudimentary spikelet showing tufts of hairs at top of pedicel.

This species resembles *Bouteloua hirsuta* more closely than any other member of the genus, but differs from it in that it is a larger and more robust plant with longer spikes and more spikelets. The rachis ends with a rudimentary spikelet and the sterile pedicel has two tufts of hair at the upper end.

Bouteloua hirsuta possibly originated from *B. pectinata* by sterilization of the outer end of the rachis and disappearance of the tufts of hair at the top of the sterile pedicel. In this change it also became a smaller plant.

Specimens examined: Fort Sill, Oklahoma, *English*, 1929; Indian Territory, *Sheldon*, 1891; Fort Worth, Texas, *Tracy*, 1902; Texas, *Nealley*, 1889; Weatherford, Texas, *Tracy*, 1902; Texas, *Germey;* Fort Worth, Texas, *Ward*, 1877; Austin, Texas, *Stiles*, 1884; Wood County, Texas, *Reverchon*, 1903; West Texas, *Mohr*, 1889; Guadalupe Mountains, *Bailey*, 1901; Mesa, Texas, *Hitchcock*, 1915; Texas, *Lindheimer;* Fort Worth, Texas, *Ruth*, 1910.

The type is in the U.S. National Herbarium, no. 1445571, collected on dry soil in a rocky mountain meadow in the Wichita Mountain region

near Fort Sill, Oklahoma, August 17, 1929 by *Burt English* (no. 71). It is somewhat immature.

The writer wishes to acknowledge the valuable help of Dr. A. S. HITCHCOCK and Mr. JASON R. SWALLEN of the National Herbarium in the loan of specimens and for aid in locating descriptions.—H. I. FEATHERLY, *Oklahoma A. & M. College, Stillwater, Okla.*

[*Accepted for publication March 19, 1930*]

NATIVITY OF THE CUCURBITS

(WITH THREE FIGURES)

DE CANDOLLE, in discussing the origin of the pumpkin, stated that "historical data do not gainsay the opinion of an American origin, but neither do they adduce anything in support of it." This statement, while no doubt true at the time it was written, is no longer correct. Thanks to the archeologists, most convincing evidence bearing upon the nativity of the cucurbits has come to light, through the recoveries made in the southwest. The writer has been privileged to make a critical study of these collections, and finds that two species of cucurbits are represented. They contain numerous fragments of the rinds and peduncles. Also in mortuary bowls recovered, cucurbit seeds have been found.

PAUL S. MARTIN, in explorations made for the Colorado State Historical Society in 1928, recovered seeds from a mortuary bowl at Ackmen, Colorado, which are identified by the writer as *Cucurbita moschata*. The seeds, although well preserved, are exceedingly fragile and contain an inert brownish mass of what was formerly the embryo. The taxonomic characters of the seed coat are well defined, and clearly show the characteristic dark, tapering, wavy margin of *C. moschata*. In the Colorado State Historical Society Museum also are cucurbit fragments, collected by A. WETHERILL. The rind is charred on one edge but is otherwise well preserved It carries the characteristic green and white stripes, and also the typical neck shape of the striped cushaw, a form of *C. moschata*. The identity of the peduncles, if considered alone, might raise a question as to whether they are *moschata* or *pepo*. The peduncle of *moschata* is extremely variable, however, and from the evidence, taken as a whole, they are probably *C. moschata* also.

Through the courtesy of Messrs. KIDDER and GUERNSEY, the writer was privileged to study their collection now in the Peabody Museum of Harvard University. From Monument Valley, White Dog cave near Kay-

enta, and from the ruins near the mouth of the Chin Lee River, Arizona, numerous recoveries of seeds of *C. moschata* have been made. Specimens from Cliff House no. 1, at Monument Valley, also include the neck with the characteristic shape and white and green stripes of the cushaw. Specimen no. A3195 from the White Dog cave is ancient material. According to KIDDER and GUERNSEY, it is from the basket-makers, a culture antedating the cliff-dwellers. They are the most ancient agricultural peoples on the North American continent of whom we have any knowledge.

FIGS. 1, 2.—Fragments of rind and peduncle of *C. moschata* recovered by WETHERILL at Mesa Verde, Colorado (courtesy of Colorado State Historical Museum).

KIDDER states that it is possible that the basket-makers as we know them lived as long ago as 1500 or 2000 years B.C.

NEIL M. JUDD, curator of American Archeology, Smithsonian Institution, states that fragments of cucurbits recovered during explorations for the National Geographic Society at prehistoric Pueblo Bonito, New Mexico, have been identified at the Bureau of Plant Industry, United States Department of Agriculture, and confirmed by COVILLE. Among these fragments both stems and seed of *C. pepo* and *C. moschata* are present; the former predominates. In the museum of the Colorado State Historical Society are specimens collected by A. WETHERILL at Mesa Verde, which are identified by the writer as *C. pepo*. L. H. BAILEY, who has made an

extended study of the cucurbits, informs me that he has received seeds from a pottery vessel taken in Arizona that are very old, and which he identifies as *C. pepo*.

In the Field Museum of Natural History are a number of interesting vases recovered by SAFFORD from the prehistoric cemeteries near Lima, Peru. They are funerary vases modeled after pumpkins, and bearing striking resemblance in details to the summer crookneck, which is a variety of *C. pepo*.

FIG. 3.—Mortuary bowl collected by SAFFORD in prehistoric cemetery near Lima, Peru (courtesy of Field Museum of Natural History); note striking resemblance to summer crookneck pumpkin, a variety of *C. pepo*, after which this vase was apparently modeled.

It is also interesting to note that JOHN K. SMALL has collected, in the everglades of Florida, a plant of unknown origin which he identifies as *C. moschata*.[1] From southern Texas, L. H. BAILEY writes regarding *C. texana*, a form allied to *C. pepo*, which has every indication of being indigenous to that region.

The evidence, therefore, seems rather conclusive that *C. moschata* and *C. pepo* are indigenous to North America. It may also be noted that, in

[1] Since the preparation of this manuscript, SMALL has described this plant as *Pepo (Cucurbita) okeechobeensis*, sp. nov.

the case of *C. maxima*, it is not represented in any of the archeological collections so far as I have been able to learn. Since it is a closely allied species it is not unreasonable to assume that it is also of American origin, and it is hoped that the archeologists may later have something to contribute in this case also.—A. T. ERWIN, *Iowa State College, Ames, Iowa.*

[*Accepted for publication February 10, 1930*]

CURRENT LITERATURE

BOOK REVIEWS

Penicillia

Mycologists are indebted to THOM for a second substantial and useful mycological and biochemical contribution. The first was the earlier volume on *Aspergilli*, and now a volume[1] on the *Penicillia* has appeared. The value of these volumes lies in the fact that they represent years of labor on the very organisms which are considered dreadful nuisances by most laboratory workers.

The volume comprises an Introduction; Characteristics of the genus (chapter I); History of *Penicillia* (chapter II); Herbaria, exsiccati, culture collections (chapter III); Generic considerations and usages (chapter IV); Culture of *Penicillia* (chapter V); Observations and descriptions of *Penicillia* (chapter VI); Physiological activities (chapter VII); Biochemistry (chapter VIII); Distribution and significance in nature and industry (chapter IX); *Penicillia* pathogenic toward man and other animals (chapter X); Classification and subdivision (chapters XI–XXV), in which it is proposed to gather the *Penicillia* into four divisions: Monoverticillata, Assymetrica, Biverticillata symmetrica, Polyverticillata symmetrica; Species of other genera described as *Penicillia* (chapter XXVI); Species identification (chapter XXVII); and Bibliography (chapter XXVIII). There is a general index and a species index.

In spite of the heroic efforts to reduce the number of species, the volume recognizes 678 species of *Penicillia*.

The volume represents an enormous labor by a most competent and experienced specialist, and goes a long way to take some of the mystery out of this genus. It is indispensable in every laboratory where microcultural work is pursued. In addition, it is a veritable *vade mecum* for specialists in fermentation, and for those dealing with the study and control of spoilage of fresh fruits and vegetables and of their products, or of animal products.—G. K. K. LINK.

A new Permian flora

A valuable contribution to our knowledge of Permian floras has been made by WHITE[2] in his description of the flora of the Hermit shale of the Grand Canyon of Arizona. The volume deals with (1) geological environment, (2) composi-

[1] THOM, C., with assistance of CHURCH, MARGARET B., MAY, O. E., and RAINES, M. A., The *Penicillia*. pp. xiii+644. *figs.* 99. Williams and Wilkins. Baltimore. 1930.

[2] WHITE, DAVID, Flora of the Hermit shale, Grand Canyon, Arizona. pp. 221. Carnegie Inst. Washington. December, 1929.

tion and relations of the Hermit flora, (3) description of the Hermit flora, and (4) the animals from the Hermit shale.

Two interesting features are strikingly apparent in the composition of the Hermit flora. First, it is largely composed of forms previously unknown, since the flora is younger than the Lower Permian floras described from other ages of North America. Second, the flora represents an aggregate, one part of which belongs to the western European or cosmopolitan flora while the other part is connected with the Gondwana flora of Asia and the southern hemisphere. In the Hermit flora the herbaceous plants, including the pteridosperms on the one hand and the shrubby or arborescent gymnosperms on the other are rather well balanced. The entire Calamarian group is absent, probably owing to seasons of too great aridity; also the failure to find any leaves of Cordaites or *Noeggerathiopsis* is notable. Members of the Araucarian stock, like *Walchia*, *Ulmannia*, and *Voltzia* are very representative. Excellent illustrations facilitate the study of this valuable contribution.—A. C. Noé.

NOTES FOR STUDENTS

X-ray mutations.—As is now well known, Muller's[3] discovery of the induction of mutations by the application of X-rays has opened a new epoch in the history of genetical research. Never has a new technique proved more rapidly prolific of instructive results.

In *Drosophila*, Oliver[4] has shown that the total number of mutations induced is directly proportional to the dosage used, when the only factor varied is the duration of treatment. There was no indication of a threshold dosage below which mutations would not be produced, thus making it easier to believe that naturally occuring mutations may owe their origin to a similar cause. For that matter, Babcock and Collins[5] have produced evidence that the frequency of naturally occurring mutations depends upon the amount of natural radiation in the vicinity. Today the weight of evidence seems to indicate that it is not the direct play of the X-rays upon the genes that is significant; but that X-rays and other forms of radiation, ionizing the materials through which they pass, thus set free rapidly moving electrons, and that the direct impacts of these electrons upon the genes cause the changes which we call mutations.

Patterson,[6] by X-raying the eggs and larvae derived from flies heterozygous for certain sex-linked genes, has produced mutation which shows up in certain

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[3] Muller, H. J., Artificial transmutation of the gene. Science **66**:84–87. 1927.

[4] Oliver, C. P., The effect of varying the duration of X-ray treatment upon the frequency of mutation. Science **71**:44–46. 1930.

[5] Babcock, E. B., and Collins, L. J., Does natural ionizing radiation control rate of mutation? Proc. Nat. Acad. Sci. **15**:623–628. 1929.

[6] Patterson, J. T., The production of mutations in somatic cells of *Drosophila melanogaster* by means of X-rays. Jour. Exp. Zoöl. **53**:327–372. 1929.

sections of the resulting bodies. The size of the mutant area depends upon the stage in ontogeny at which the treatment was applied. PATTERSON has also produced reverse mutations, showing that the original mutation induced by X-ray was more than a simple destruction, as some have suggested.

Time relations in the X-raying of males have been studied by HARRIS,[7] and by HANSON and HEYS.[8]

In addition to the considerable investigation of induced gene mutations that is now being carried on, MULLER and PAINTER,[9] and also DOBZHANSKY,[10] are conducting parallel genetical and cytological investigations of the redistribution of chromosome parts that is induced by X-ray. The results constitute the most striking comfirmation yet provided of the chromosome theory of heredity and the linear arrangement of genes; for translocations of sections of the various linkage groups are accompanied by corresponding translocations of visible chromosome segments. Although the genes maintain their previous sequence in the line, the visible size of the translocated segments does not correspond perfectly with the simple map distances assigned to the linkage groups from genetical results. As MULLER himself had maintained, the regions of the linkage groups near to the points of spindle fiber attachment on the chromosomes are actually longer than the maps indicate; for the frequency of crossing over is systematically reduced in these regions.

Among plants, GOODSPEED[11] had early secured results from X-raying *Nicotiana*. GAGER and BLAKESLEE[12] now report mutations of various types following the treatment of *Datura* with radium rays.

About the most instructive plant work has been done by STADLER,[13] who was

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[7] HARRIS, B. B., The effects of aging of X-rayed males upon mutation frequency in *Drosophila*. Jour. Hered. **20**:299–302. 1929.

[8] HANSON, F. B., and HEYS, FLORENCE, Duration of the effects of X-rays on male germ cells in *Drosophila melanogaster*. Amer. Nat. **63**:511–516. 1929.

[9] MULLER, H. J., and PAINTER, T. S., The cytological expression of changes in gene alignment produced by X-rays in *Drosophila*. Amer. Nat. **73**:193–200. 1929.

———, Parallel cytology and genetics of induced translocations and deletions in *Drosophila*. Jour. Hered. **20**:287–298. 1929.

[10] DOBZHANSKY, T. L., Genetical and cytological proof of translocations involving the third and the fourth chromosomes of *Drosophila melanogaster*. Biol. Zentralbl. **49**:408–419. 1929.

[11] GOODSPEED, T. H., Cytological and other features of variant plants produced from X-rayed sex cells of *Nicotiana tabacum*. BOT. GAZ. **87**:563–582. 1929.

[12] GAGER, C. S., and BLAKESLEE, A. F., Chromosome and gene mutations in *Datura* following exposure to radium rays. Proc. Nat. Acad. Sci. **13**:75–79. 1927.

[13] STADLER, L. J., Some genetic effects of X-rays in plants. Jour. Hered. **21**:3–19. 1930.

———, Genetic effects of X-rays in maize. Proc. Nat. Acad. Sci. **14**:69–175. 1928.

———, Mutations in barley induced by X-rays and radium. Science **68**:186–187. 1928.

independently at work along these lines while MULLER was performing his first experiments. X-raying barley seeds frequently induced gene mutation in one of the primordia of the embryo. Of the resulting tillers, one was mutant, and progenies of this one head revealed only the mutant character. Fifty-eight gene mutations were found among 2800 head progenies, as against no mutations in 1500 head progenies of the untreated control. All observed mutations were recessive; 90 per cent of them involved seedling characters, and most of these showed chlorophyll abnormalities.

Frequency of induced mutation increased in direct proportion to X-ray dosage, which was varied by changing the duration of the treatment. At the same dosage germinating seeds mutated four times as frequently as did dormant seeds, but the latter would withstand twenty times as heavy a dose without being killed.

In barley, with its seven chromosome pairs, and in those species of wheat and oats which also have seven, the induced mutation rate was about the same. Species of wheat and oats with 14 chromosome pairs, and those with 21 pairs, responded much less frequently if at all. This difference is to be expected if the species with higher chromosome numbers, having been derived originally from those with the lower, contain numerous sets of duplicate or triplicate genes. In such cases induction of single recessive gene mutations would not be detectable.

Here too X-raying also induces chromosome aberrations. Maize, in which the linkage groups are rather well known, lends itself particularly well to this study. If young developing endosperms of formula C-Sh-Wx, c-sh-wx, c-sh-wx are X-rayed, endosperm patches which are colorless, shrunken, waxy, will appear, probably through the loss of the C-Sh-Wx chromosome. The size of these patches depends upon the stage of development of the endosperm at which the X-raying occurred. Comparable effects upon the embryo are revealed by the condition of plants resulting from such grains.

STADLER feels that profitable practical application of this new technique will be limited to those situations in which hybridization is not a feasible method of plant improvement. A good example of this is the induction of bud mutations in fruit trees.

In a delightful popular article, MULLER[14] has thrown the light of all these new discoveries upon the process of evolution.—M. C. COULTER.

STADLER, L. J., The rate of induced mutation in relation to dormancy, temperature, and dosage. Anat. Rec. **41**:77. 1928.

———, Chromosome number and the mutation rate in *Avena* and *Triticum*. Proc. Nat. Acad. Sci. **15**:876–881. 1929.

[14] MULLER, H. J., The method of evolution. Scientific Monthly **29**:481–505. 1929.